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Search Results -

Terms	Documents
L7 same (copy with number$)	41

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DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=NO; OP=OR			
L8	L7 same (copy with number$)	41	L8
L7	(tunicamycin or antibiotic) with resistance with increas$	526	L7
L6	l2 and (increas$ with concentration$ with antibiotic$)	4	L6
L5	l2 and (titrat$ same antibiotic$)	1	L5
L4	L2 and (high with copy with number$)	111	L4
L3	L2 and (high with copy with number$ with indicator$)	0	L3
L2	L1 same vector$	698	L2
L1	multiple same insert$ same screen$	2384	L1

END OF SEARCH HISTORY

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Search Results -

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L7 same (copy with number$)	41

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L2	L1 same vector$	698	L2
L1	multiple same insert$ same screen$	2384	L1

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S7	435	S6 AND (MULTIPLE OR SECOND) AND INSERT? AND VECTOR?
S8	100	S7 AND (GALACTOSIDASE OR ANTIBIOTIC?)
S9	86	RD (unique items)
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>>>KWIC option is not available in file(s): 399

12/3,K/1 (Item 1 from file: 71)
DIALOG(R)File 71:ELSEVIER BIOBASE

00361738 96036691
Conditionally replicative and conjugative plasmids carrying lacZalpha for cloning, mutagenesis, and allele replacement in bacteria
Metcalf W.W.; Jiang W.; Daniels L.L.; Kim S.-K.; Haldimann A.; Wanner B.L.
ADDRESS: B.L. Wanner, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, United States
Journal: Plasmid, 35/1 (1-13), 1996, United States
PUBLICATION DATE: 19960000
CODEN: PLSMD
ISSN: 0147-619X
DOCUMENT TYPE: Article
LANGUAGES: English SUMMARY LANGUAGES: English

We describe several new cloning *vectors* for mutagenesis and allele replacement experiments. These plasmids have the R6Kgamma DNA replication origin (oriR(R6Kgamma)) so they replicate only in bacteria supplying the Pi ...

...transfer origin (oriT(RP4)) so they can be transferred by conjugation to a broad range of bacteria. Most of them encode lacZalpha for blue-white *color* *screening* of colonies for ones with plasmids carrying *inserts*, as well as the f1 DNA replication origin for preparation of single stranded DNA. Particular plasmids are especially useful for allele replacement experiments because they...

...pir host (via conjugative transfer, transformation, or electroporation), and integrants with the plasmid recombined into the chromosome via homologous sequences are selected using a plasmid *antibiotic* resistance marker. Plasmid- free segregants with an allele replacement can be subsequently selected as Tet(S) or Suc(R) recombinants. A number of additional features (including the presence of *multiple* cloning sites flanked by T3 and T7 RNA polymerase promoters) make these plasmids useful as general cloning *vectors* as well.

CLASSIFICATION CODE AND DESCRIPTION:

...*Vectors*

12/3,K/2 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

04329695 EMBASE No: 1990217758
A novel phosphate-regulated expression *vector* in Escherichia coli
Su T.-Z.; Schweizer H.; Oxender D.L.
Dept. of Biological Chemistry, University of Michigan,Ann Arbor, MI 48109-0606 United States
Gene (GENE) (Netherlands) 1990, 90/1 (129-133)
CODEN: GENED ISSN: 0378-1119
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

A novel phosphate-regulated expression *vector* in Escherichia coli

...Escherichia coli was cloned into a small multicopy plasmid pTER5, a derivative of pBR322, between the transcription terminators rpoCt and t(L1). The resulting expression *vector*, pPH3, permits convenient *insertion* of structural genes containing their own translational-initiation regions, into the *multiple*-cloning site derived from the pUC19 plasmid. The efficiency and regulatory properties of p(ugp) were *measured* using xylE and lacZ as reporter genes, which code for the corresponding enzymes catechol-2,3-dioxygenase (C23O) and beta-*galactosidase* (betaGal), respectively. Enzyme activities were virtually completely repressed in the presence of excess inorganic phosphates (P(i)) and high *concentrations* of glucose. Maximal induction was observed at limiting P(i) (<0.1 mM) and normal levels of glucose (0.2-0.4%). The maximum expression...

...cellular protein as judged by laser densitometry following sodium dodecyl sulfate-polyacrylamide-gel electrophoresis. These results suggest the usefulness of the p(ugp) in expression *vectors* for strong, but controlled, expression of cloned genes in E. coli. This P(i) controlled *vector* can be adapted to large-scale fermentation by using P(i)-limiting growth conditions.

12/3,K/3 (Item 1 from file: 370)
DIALOG(R)File 370:Science

00508288 (USE 9 FOR FULLTEXT)
dMi-2, a Hunchback-Interacting Protein That Functions in Polycomb Repression
Kehle, Johannes; Beuchle, Dirk; Treuheit, Susanne; Christen, Bea; Kennison, James A.; Bienz, Mariann; Mueller, Juerg
J. Kehle, D. Beuchle, S. Treuheit, Max-Planck-Institut fuer Entwicklungsbiologie, Spemannstrasse 35/III, 72076 Tuebingen, Germany. B. Christen, M. Bienz, and J. Mueller, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK. J. A. Kennison, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-2785, USA.
Science Vol. 282 5395 pp. 1897
Publication Date: 12-04-1998 (*981204*) Publication Year: 1998
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2173

(THIS IS THE FULLTEXT)
(*981204*)
...Text: may act as a molecular link between the Hb repressor and PcG proteins, we used Hb protein as a bait in a yeast two-hybrid *screen*.

...

...the six cDNAs interacted exclusively with Hb (Fig. 1 A). Among these proteins, the hip76 clone product exhibited the strongest interaction with Hb. We isolated *multiple* cDNA clones (B10) that span a complete open reading frame (ORF) encoding a 1982-amino acid protein with high sequence similarity to the human autoantigen...

...To map the dMi-2-interacting domain in Hb, we generated Hb fragments and tested them for dMi-2 interaction in yeast two-hybrid *assays* (Fig. 1B). dMi-2 interacted very strongly with sequences overlapping the D domain, a stretch of amino acids that is conserved between Hb proteins of...

...for repression of BXC genes (B19) . Our interaction tests (Fig. 1B) show that this protein portion of Hb interacts with dMi-2. In vitro binding *assays* with bacterially expressed dMi-2 and Hb proteins confirmed that these proteins bind directly to each other (B20) . Thus, dMi-2 binds to a portion...

...In situ hybridization to polytene chromosomes revealed that dMi-2 maps to subdivision 76D (B21) . In a *screen* for zygotic lethal mutations in this region, we identified five complementation groups (B21...

...dMi-2 homozygotes survived until the first or *second* larval instar. Mutant embryos and larvae showed no obvious mutant phenotypes. Specifically, expression of BXC genes such as Ultrabithorax (Ubx) and Abdominal-B (Abd-B...for both Pc and dMi-2 mutations showed more extensive derepression (Fig. 3B). Furthermore, derepression of the HOX gene Sex combs reduced (Scr) in the *second* and third leg discs of Pc heterozygotes results in the formation of a first leg structure, the sex comb, on the *second* and third legs (B25) . The extent of this homeotic transformation reflects the number of cells that misexpress Scr protein. We found that this homeotic transformationYeast two-hybrid *assay*. Six Hb-interacting proteins (hip) were isolated. Parentheses denote number of times isolated. Blue *color* intensity of yeast colonies grown on X-gal plates indicated strength of interaction [- and + signs in (A) and (B)]. Only hip57, hip66, and hip76 exclusively...

...activation domain (AD) fusion. With the exception of LexA-Hb(2-487) and LexA-Hb(2-344), these fusions did not autoactivate transcription (NONE). Repression *assays* (B29) demonstrated that all LexA-Hb fusion proteins bind to LexA operator sites in yeast nuclei. The D domain (black box) together with sequences directly...

...2. F1, finger domain 1; D, D domain; F2, finger domain 2. (C) Lesions present in dMi-2 alleles. dMi-2.sup(4) shows an *insertion* of 4 base pairs after codon 398 that results in a frameshift and consequently a predicted premature termination within the first PHD finger domain. In...

...dMi-2/Pc transheterozygotes (on average, three times as many cells express Ubx). (C) Homeotic transformations due to derepression of Scr. Each number in the *second* and third columns is the total number of sex comb teeth on *second* and third legs of 20 sibling males of the genotypes indicated. The ratio of the numbers in the *second* and third columns shows the enhancement caused by the mutation X. All dMi-2 mutations enhance the transformation comparable to a Pcl mutation. Mutations in...

References and Notes:

...9. A Drosophila cDNA expression library was screened with a LexA-Hb expression *vector* containing the full Hb ORF (Hb.inf(2-789)). 2 x 10.sup(6) primary yeast transformants were harvested, and about 2 x 10.sup...Pc.sup(XT109) larvae were identified with appropriately marked balancer chromosomes. Standard procedures were used for staining with antibodies to Ubx, Abd-B, and (beta) -*galactosidase*. ;

12/3,K/4 (Item 2 from file: 370)

00508121 (USE 9 FOR FULLTEXT)

Tankyrase, a Poly(ADP-Ribose) Polymerase at Human Telomeres

Smith, Susan; Giriat, Izabela; Schmitt, Anja; de Lange, Titia
The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

Science Vol. 282 5393 pp. 1484
Publication Date: 11-20-1998 (*981120*) Publication Year: 1998
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2403

(THIS IS THE FULLTEXT)
(*981120*)

...Text: To identify additional telomere-associated proteins, we used a yeast two-hybrid *screen* with human TRF1 as bait (B5) (B6) . This *screen* yielded two overlapping partial cDNAs (TR1L-4 and TR1L-12) (Fig. 1A). A full-length testis cDNA isolated with TR1L-4 encoded an open reading...

...B10) . First, tankyrase, like all ankyrins, contained 24 copies of the ANK motif, whereas other ANK repeat-containing proteins typically have 4 to 8 repeats. *Second*, the ANK repeats in tankyrase and the ankyrins shared characteristic sequence features, such as the presence of a hydrophobic amino acid at position 3 and...

...ribose) onto a protein acceptor using nicotinamide adenine dinucleotide (NAD.sup(+)) as a substrate (B9) . The catalytic domain of PARP consists of secondary structure units (*multiple* (beta) strands and one a helix) (Fig. 2C) that form a cavity known as the NAD.sup(+)-binding fold, a tertiary structure that is also...

...Northern (RNA) blot analysis revealed that *multiple* tankyrase mRNAs (B13) were ubiquitously expressed in human tissues, with the highest amounts detectable in testis (Fig. 3A). TRF1 and TRF2 transcripts show a similar...To investigate whether tankyrase has PARP activity, we tested baculovirus-derived recombinant protein in an *assay* that *measures* the addition of radiolabeled ADP-ribose to protein acceptors with [.sup(32)P]NAD.sup(+) used as a substrate (B17) . Incubation of tankyrase in the...

...NAD.sup(+) produced .sup(32)P-labeled species that comigrated with tankyrase, suggesting that tankyrase has the ability to ADP-ribosylate itself (Fig. 4A). Higher *concentrations* of NAD.sup(+) (0.04 to 1 mM) yielded much larger products, likely reflecting the addition of poly(ADP-ribose) to tankyrase. The generation of ADP-ribosylated tankyrase depended on the *concentration* of tankyrase (Fig. 4A), was eliminated by heat inactivation of the enzyme, and could be immunoprecipitated with anti-tankyrase (Fig. 4B) (B18) , indicating that the...

...Tankyrase also has the ability to modify TRF1. At low NAD.sup(+) *concentration* (1.3 (mu) M), the ADP-ribosylated products comigrated with TRF1, whereas at higher NAD.sup(+) *concentrations* (0.04 to 1 mM), the slower and variable mobility of the labeled products suggested poly(ADP-ribosyl)ation of TRF1 (Fig. 4A). Inspection of...

...weight species upon tankyrase-mediated TRF1 modification, indicating that only a small fraction of the TRF1 in the reactions was modified even at high tankyrase *concentrations*. Thus, tankyrase is likely to function as a processive PARP under these conditions. TRF2 is not a substrate for modification in vitro, as expected from...

...The effect of tankyrase on the telomeric DNA binding activity of TRF1 was determined by an in vitro gel-shift *assay* with the use of a double-stranded array of [TTAGGG].inf(12) as a probe (B20) . TRF1 binds to

DNA as a homodimer, and several such dimers can occupy one [TTAGGG].inf(12) molecule at high TRF1 *concentrations* (B6) (Fig. 4E). When TRF1 was incubated with baculovirus-derived tankyrase in the absence of NAD.sup(+), a slight stimulation of the TRF1 DNA binding activity occurred, resulting in the formation of higher order complexes, especially at high tankyrase *concentrations*. However, this stimulation of TRF1 also occurred with total insect cell protein and was therefore unlikely to represent a specific effect of tankyrase. A similar...Domain structure of tankyrase and two-hybrid interaction with TRF1. (A) Schematic representation of the structure of tankyrase and TRF1. Lines below the schematic indicate *inserts* of the two-hybrid plasmids (TR1L-4 and TR1L-12) and a plasmid used to generate recombinant protein for antibody production (ANK2). HPS, region containing...

...homology to the catalytic domain of PARP; Myb, Myb-type DNA binding motif; D/E, acidic domain rich in Glu and Asp. (B) Two-hybrid *assay* for the tankyrase interaction domain in TRF1. (beta) -*Galactosidase* *concentrations* (Miller units; average of three independent transformations) were *measured* for strains expressing the indicated fusion proteins (B6) . GAD, GAL4 activation domain...

...tankyrase. (A) Predicted amino acid sequence of tankyrase with an alignment of the 24 ANK repeats. Dashes indicate gaps, and sequences to the right indicate *insertions* that occur after the underlined amino acid in each line. Light shading indicates a match to the ANK repeat consensus, and darker shading is a...

...ADP-ribosylation activity is intrinsic to tankyrase. Tankyrase was immunoprecipitated with preimmune serum or anti-tankyrase (a-Tankyrase) as indicated and incubated in a PARP *assay* with [.sup(32)P]NAD.sup(+). The products were detected by autoradiography (B18) . (C) Tankyrase is inhibited by the PARP inhibitor 3AB. Reactions containing 4...and analyzed by autoradiography (left) or immunoblotted with monoclonal antibody 10H to poly(ADP-ribose) (B17) (right). (E) Tankyrase inhibition of TRF1. A gel-shift *assay* for the TTAGGG repeat-binding activity of TRF1 was performed with a duplex [TTAGGG].inf(12) DNA as a probe. Binding reactions contained the components indicated above the lanes. Tankyrase *concentration* was varied from 200 to 2.5 ng per 20- (mu) l incubation in threefold dilution steps (triangle). TRF1 was either present at 13 ng...

References and Notes:

...of a human fetal liver two-hybrid library (Clontech, Palo Alto, CA) created in pGad10. Of 13 positives, 12 contained an identical 2.4-kb *insert* (TR1L-4) and one had a 1-kb *insert* (TR1L-12), which was contained within TR1L-4. DNA sequence analysis indicated that TR1L-4 was a partial cDNA...

...7. A full-length tankyrase cDNA TT20 containing a 4134-nucleotide (nt) *insert* was isolated as follows. First, two overlapping cDNAs, 32 and 21, encompassing 8901 nt encoding amino acids 235 to 1327 were isolated from a HeLa...

...library isolates, TT7 and TT9 (GenBank accession numbers AF082558 and AF082559), indicated that they were similar to TT20 along their length, but each had an *insertion* of ~100 nt [TT7, *insertion* after amino acid 640 (in ANK repeat 14), and TT9, *insertion* after amino acid 881 (in ANK repeat 21)]. Both *insertions* contained stop codons ...enhanced chemiluminescence (Amersham). For generation of antibody to tankyrase (anti-tankyrase), the Ank2 fragment representing amino acids 973 to 1149 of tankyrase was fused to *vector* pET-22b(+) (Novagen) and expressed in Escherichia coli. The protein was isolated in inclusion bodies and used to immunize one rabbit. The resulting immune serum...

...of DNA. To make baculovirus-derived protein, we generated an NH.inf(2)-terminally (His).inf(6)-tagged version of human tankyrase in the expression *vector* pFastBac HTb (Gibco BRL) and used this construct to generate a recombinant plasmid in DH10Bac E. coli. The recombinant DNA

was used to transfect SF21...

12/3,K/5 (Item 3 from file: 370)
DIALOG(R)File 370:Science

00507634 (USE 9 FOR FULLTEXT)
Controlling Gene Expression in Living Cells Through Small Molecule-RNA Interactions
Werstuck, Geoffrey; Green, Michael R.
Howard Hughes Medical Institute, Program in Molecular Medicine, University of Massachusetts Medical Center, 373 Plantation Street, Suite 309, Worcester, MA 01605, USA.
Science Vol. 282 5387 pp. 296
Publication Date: 10-09-1998 (*981009*) Publication Year: 1998
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 1374

(THIS IS THE FULLTEXT)
(*981009*)

...Abstract: it is shown that small molecule aptamers also bound their ligand in vivo, enabling development of a method for controlling gene expression in living cells. *Insertion* of a small molecule aptamer into the 5 (prime) untranslated region of a messenger RNA allowed its translation to be repressible by ligand addition in...

...Text: We selected RNA aptamers that specifically bound to the related aminoglycoside *antibiotics* kanamycin A and tobramycin (Fig. 1 A) (B5) . We analyzed the ability of these aptamers to function in vivo by expressing them in Escherichia coli...

...tob1 was negligible (Fig. 1C). In the presence of 10 (mu) M tobramycin, bl-tob1 grew to saturation, and bl-kan1 grew to a subsaturating *concentration* (Fig. 1C) (B7) . Increasing the number of aptamers in the expression *vector* from one to three enhanced growth in the presence of *antibiotic* (Fig. 1D). Thus, a specific drug-resistant phenotype was conferred by expression of an aminoglycoside aptamer (B8) , which demonstrates a small molecule-aptamer interaction in...

...We constructed an mRNA that contained three copies of the tob aptamer *inserted* in the 5 (prime) UTR of RSETA (tob3-RSETA). In vitro translation (B11) of the control RSETA mRNA was unaffected by all *concentrations* of tobramycin or kanamycin tested, whereas addition of tobramycin inhibited in vitro translation of the tob3-RSETA mRNA in a dose-dependent fashion (Fig. 2) (B12) . In vitro translation of the tob3-RSETA mRNA was not inhibited by comparable *concentrations* of kanamycin A, which is not recognized by the tob aptamer...
...expression in vivo. Because aminoglycosides are relatively impermeable to the plasma membrane, can be cytotoxic, and have a general inhibitory effect on translation at high *concentrations* (B13) , we used a cell-permeable small molecule as the translation regulator...

...two of which-H10 and H19-are shown (Fig. 3B). Both H10 and H19 bound to an H33258 affinity column and required a relatively high *concentration* (25 mM) of free H33258 for elution (Fig. 3C) (B16...

...To demonstrate that the H33258 aptamer could be used to regulate translation, we *inserted* one copy of H10 and H19 in tandem into the 5 (prime) UTR of RSETA. Addition of H33258 inhibited in vitro translation of H2-RSETA...

...To test whether this small molecule-aptamer interaction could be used to control gene expression in vivo, we *inserted* one copy of H10 and H19 into

the 5 (prime) UTR of a mammalian (beta) -*galactosidase* expression plasmid, SV (beta) Gal (Promega), generating the construct SVH2 (beta) gal. Chinese hamster ovary (CHO) cells were cotransfected with SVH2 (beta) Gal or, as a control, the parental *vector* SV (beta) Gal and a luciferase reporter gene to provide an internal control (B17) ..After transfection, cells were grown for 24 hours in the presence of 0, 5, or 10 (mu) M H33342 and analyzed for (beta) -*galactosidase* and luciferase activities (B18...

...with the in vitro translation data of Fig. 3D. Expression of the luciferase reporter was also not inhibited by H33342 (B19) . However, H33342 reduced (beta) -*galactosidase* activity from SVH2 (beta) Gal by greater than 90% in a dose-dependent fashion (Fig. 3E...

...Figure F1
Caption: Selective interaction between aminoglycosides and aminoglycoside aptamers in vivo. (A) Structures of aminoglycoside *antibiotics* and their aptamers. Consensus aptamers were identified after 10 to 12 rounds of selection. (B to D) Growth curves. Overnight cultures of E. coli BL-21 transformed with plasmids expressing RSETA, tob1, tob3, kan1, or kan3 were diluted 1:100 into medium containing the indicated *concentration* of aminoglycoside *antibiotic*. Optical density (660 nm) was *measured* at fixed intervals over 8 hours of growth at 37.Deg.C. (B) Growth in the absence of drug. (C) Growth in the presence of...

...In vivo expression. H33258 aptamers H10 and H19 were cloned in tandem into the 5 (prime) UTR (Sfi I-Avr II sites) of a (beta) -*galactosidase* reporter gene (SV (beta) gal; Promega) to generate SVH2 (beta) gal. CHO cells were cotransfected with 1 (mu) g of SV (beta) gal or SVH2 (beta) gal and 1 (mu) g of a luciferase expression *vector* (pGL3). Cells were grown in the presence of 0, 5, or 10 mM H33342. Twenty-four hours after transfection, cell extracts were prepared and (beta) -*galactosidase* and luciferase activities were determined...

References and Notes:
...aptamer were cloned into the Nde I site (one copy) or Nde I and Bsa I sites (three copies) of T7 RNA polymerase-driven expression *vector* pRSETA (Invitrogen) and transformed into a bacterial strain containing an isopropyl (beta) -d-thiogalactopyranoside (IPTG)-inducible T7 RNA polymerase. Bacterial strains were grown in liquid culture overnight, induced with 0.1 mM IPTG for 1 hour, and then diluted into medium containing *antibiotic* and 0.1 mM IPTG...

...8. None of the strains exhibited increased resistance to the unrelated *antibiotics* tetracycline and gentamicin...

...12. Translation repression was more efficient with *multiple* aptamers than with a single aptamer. Repression was also more efficient when the aptamers were positioned near the 5 (prime) end of the mRNA...the cells. Twenty-four hours after transfection cells were harvested and cell extracts were prepared. Cell extracts were normalized for total protein (Bradford assay). (beta) -*Galactosidase* and luciferase activities in the extracts were determined relative to standard curves generated with the purified (beta) -*galactosidase* and luciferase enzymes (Promega...
...19. The parental expression *vector* SV (beta) Gal was also not inhibited by 5 or 10 (mu) M H33342...

12/3,K/6 (Item 4 from file: 370)
DIALOG(R)File 370:Science

00507103 (USE 9 FOR FULLTEXT)
Genetic Dissection of a Mammalian Replicator in the Human (beta) -Globin Locus
Aladjem, Mirit I.; Rodewald, Luo Wei; Kolman, John L.; Wahl, Geoffrey M.
Gene Expression Laboratory, The Salk Institute, San Diego, CA 92037, USA.

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Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 3663

(THIS IS THE FULLTEXT)
(*980814*)

...Text: DNA (B8) , failure to license transfected DNA for initiation before it integrates (B9) , or greater complexity of mammalian replicators, exceeding the cloning capacity of the *vectors* used (B7) . Alternatively, replication initiation may be determined by a specific nuclear structure established within a defined cell cycle interval rather than by DNA sequence...
...locus that encompasses the IR in ectopic sites in the simian (Cercopithecus aethiops) genome (Fig. 1A). A polymerase chain reaction (PCR)-based nascent strand abundance *assay* (Fig. 1B) (B15) revealed that short, newly replicated nascent strands were generated from the (beta) -globin IR in both sites (Figs. 2 A and 3...

...strands are produced from regions within which DNA replication initiates (B11) . By contrast, short nascent strands were not generated at detectable levels from the (beta) -*galactosidase* ((beta) -Gal) gene sequences located 5 kb 3 (prime) of the IR (Figs. 2A and 3). Globin sequences were also present in preparations of small nascent strands obtained from cells in which the transferred IR was *inserted* into a *second* ectopic location (B16...at different locations within the IR. First, we isolated short (1 to 2 kb) nascent strands and determined the relative abundance of globin sequences using *multiple* primer pairs arrayed across the transferred 8-kb IR. All primer pairs were equally efficient in amplifying genomic DNA (Fig. 3, A and B). If...

...In the *second* strategy, we isolated nascent strands 0.5 to 8.0 kb in size and determined the abundance of globin sequences within them. Because the abundance...
...greater by at least two orders of magnitude than the abundance of nonorigin sequences in similar preparations (Fig. 3A), we used a semiquantitative approach to *measure* the representation of DNA sequences in the size-fractionated samples. Within an initiation region, it is predicted that sequences near an initiation site should be...

...an initiation region should be present in long, but not short, nascent strands. Small, size-fractionated nascent strands were amplified in the presence of constant *concentrations* of competitor DNA to estimate the relative abundance of IR sequences within the preparation. The h (beta) G sequences were abundant in short and long...

...is similar to the pattern of nascent strands observed in the native h (beta) G locus (B11) and when the IR was integrated into a *second*, independent genomic site in simian cells (B16) . Similar nascent strand length patterns have been reported to be produced in yeast (B21) (B22) and in Chinese...

...Fig. 4A, IR-HS) of the 8-kb IR. However, deletion of both distal sequences reduced initiation to a level no longer detectable by the *assay* used (Fig. 4A, IR-PS). Deletion of the "core" region of ~2.6 kb from the 8-kb IR (IR-HP/SH) also abolished initiation...locations, this function may be subserved by other cis-acting sequences. Because our FLP recombination target (FRT) integration sites were selected for the expression of *antibiotic* resistance markers, it is reasonable to assume that these sites are in open chromatin. Thus, replicator activity in mammalian cells may be defined in terms...

...of a (beta) -Gal cassette interrupted by a FLP recombination target (FRT), were used as acceptor cell lines. Putative replicators were cloned

into a shuttle *vector*, SFV, containing an identical FRT and LoxP site, the target for the Cre recombinase (B13) . Transfection of the SFV *vector* , containing the putative replicator, in the presence of excess FLP resulted in integration of the *vector* at the FRT site between the promoter and the open reading frame (B13) . Site-specific integration was selected for hygromycin resistance and *screened* by negative (beta) -Gal staining. For *insertion* of LCR sequences 5 (prime) into the transferred origin, a mini-LCR (B31) was cloned into the SFV shuttle *vector* and transfected into the acceptor lines in the presence of excess Cre. The establishment of a cell line containing a single-copy site-specific integration of the desired sequence was verified by Southern (DNA) blot analysis. This strategy enables all variants of the replicator (for example, deletions and mutations) *inserted* into the SFV shuttle *vector* and transfected into the same acceptor cell line to be *assayed* for the ability to initiate DNA replication in an identical genetic background and chromosomal environment. Selective markers: hyg, hygromycin resistance; Bst.sup(r), blasticidin resistance. (B) An *assay* for initiation of DNA replication. Initiation of DNA replication from the integration site was tested by the nascent strands abundance *assay* (B40) . Cells are pulse-labeled with BrdU, lysed gently, and small DNA strands isolated after fractionation through a sucrose gradient. Newly replicated DNA strands are ...

...by two PCR primer pairs within the transferred IR and one PCR primer pair 3 (prime) to the IR in the presence of increasing competitor *concentration* (ranging from 0.001 to 1.0 pg). G, genomic DNA; C, competitor. Hatched bar, position of the (beta) -globin origin as in Fig. 2Figure Removed

Figure F4
Caption: . Sequences required for replicator activity. Deletions of the transferred IR were cloned into SFV and *inserted* into the acceptor cell lines, then *assayed* for the abundance of IR sequences in nascent strands as described in the legend to Figs. 1 and 3B. (A) A representative analysis with h...
...of the Pme I site at position 61,110 to the Hind III site at position 67,377). All the IR fragments described above were *inserted* into the same chromosomal sites, and at least two sets of nascent strands were analyzed for each fragment. Similar results were obtained when the abundance of nascent strands in a *second* site was analyzed (B16...

References and Notes:
...reactions were performed with Taq polymerase (Boehringer Mannheim), and the salt conditions were optimized for each primer pair with the Invitrogen PCR optimizer kit. Competitor *concentrations* were determined by reading the absorbency of the cloned fragments at 260 nm. Because the transferred IR was *inserted* in simian cells, all the primer pairs were *assayed* for selective amplification of human, but not simian, DNA before use in this *assay*. PCR reactions with primers only, or with genomic or competitor DNA with no primers, yielded no product...

12/3,K/7 (Item 5 from file: 370)
DIALOG(R)File 370:Science

00506965 (USE 9 FOR FULLTEXT)
Regulation of a Transcription Factor Network Required for Differentiation and Metabolism
Duncan, Stephen A.; Navas, M. Angeles; Dufort, Daniel; Rossant, Janet; Stoffel, Markus
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Language: English
Section Heading: Reports
Word Count: 2400

(THIS IS THE FULLTEXT)
(*980731*)

Text: Hepatocyte-specific gene expression is controlled primarily at a transcriptional level and relies on the activity of *multiple* transcription factors including HNF-1, CCAAT/enhancer binding protein (C/EBP), HNF-3, HNF-4, and HNF-6 (B1) . Our current understanding of transcriptional regulation...

...B1) . Although these approaches have provided useful information about tissue-specific regulation of gene expression, it is important to note that such procedures do not *measure* transcriptional regulation within a native chromosomal context. This is significant, given that HNF-3 proteins have been shown to modify the nucleosomal organization of the...

...DNA binding domain and the COOH-terminal transcriptional transactivation domains by homologous recombination in ES cells (B10) . In addition, the Escherichia coli LacZ gene was *inserted* into exon 2 of HNF-3a, producing a gene fusion that allowed us to follow gene expression from the HNF-3a promoter in embryoid bodies (EBs) and embryos (Fig. 1). Three independent HNF-3a -/- lines were then produced by culturing the HNF-3a +/- ES cells in high *concentrations* of G418 medium, as described (B11...

...Steady-state mRNA *concentrations* of numerous genes expressed in the VE were *measured* in 21-day-old HNF-3a +/+, +/-, and -/- EBs by reverse transcription polymerase chain reaction (RT-PCR) (Fig. 2). Hypoxanthine phosphoribosyltransferase (HPRT) primers were used to show that each sample contained equivalent amounts of mRNA. Furthermore, mRNA *concentrations* of VE markers GATA-4 and HNF-1 (beta) (B12) were similar, demonstrating that each sample contained equal amounts of VE. As expected, no HNF...

...aldolase B and l-pyruvate kinase, which are putative targets for HNF-3a, were upregulated three-to eightfold in HNF-3a -/- EBs. In addition, mRNA *concentrations* of HNF-4a and HNF-1a were increased, whereas HNF-3 (beta) and -3 (gamma) remained relatively unaffected. LacZ expression was similar in HNF-3a...and gluconeogenesis by HNF-3 and HNF-4 led us to investigate whether HNF-3a/HNF-3 (beta) ratios were affected by insulin (B19) . We *measured* steady-state *concentrations* of HNF-3a and HNF-3 (beta) mRNAs in day14 EBs that were cultured for 24 hours in serum-free medium containing 0, 5, or...A) Expression of LacZ in HNF-3a -/- ES cell EBs. HNF-3a -/- EBs were cultured for 14 days in suspension and then stained for (beta) -*galactosidase* activity. LacZ expression is restricted to the VE (arrow). (B) HNF-3a -/- ES cell-derived embryos were produced by tetraploid aggregation as described in (B16) . Embryos were harvested at E8.5 and stained for expression of lacZ. All embryos were indistinguishable from wild types and exhibited (beta) -*galactosidase* activity in the floorplate of the neural tube and notochord (F/N) as well as gut (G) and developing liver (L...
...3a +/+ (R1; lane 1), HNF-3a +/- (A13 and A7; lanes 2 and 6), and HNF-3a -/- (A8, A10, and A11; lanes 3 to 5) were *assayed* for expression of mRNAs from HPRT; GATA-4; HNF-1a, -3a, -3 (beta) , -3 (gamma) , and -4a; albumin (Alb); apolipoproteins (APo) AI, AII, AIV, B...

...Figure F3
Caption: HNF-3 (beta) -dependent gene expression in EBs of wild-type, heterozygous, and HNF-3 (beta) null ES cells. Steady-state mRNA *concentrations* of putative target genes were *measured* by RT-PCR (Fig. 2) (B4) . TTR, Transthyretin...

...grown in the presence of hygromycin (0.4 mg/ml) for 8 days and three resistant lines were used to generate EBs. Steady-state mRNA *concentrations* were *measured* by RT-PCR in 14-day-old EBs.

Concentrations of HNF-3a mRNA were moderate in lines a3 and a5 and highest in line a4. The rescue of HNF-3a expression in HNF-3or 50 mM insulin for 24 hours. Steady-state mRNA *concentrations* of HPRT, GATA-4, HNF-3a, HNF-3 (beta) , HNF-4a, Aldo-B, and L-PK mRNA were determined by RT-PCR...

References and Notes:
...10. The HNF-3a targeting *vector* was constructed by deleting the DNA binding and transactivation domains between the Afl III and Nhe I sites in exon 2. The E. coli lacZ...

...digested with Hind III-Eco RV and blots were probed with HNF-3a genomic DNA, which lay 5 (prime) to sequences present in the targeting *vector*. The wild-type allele produced a 7.5-kb restriction fragment, whereas the targeted allele produced a 4.5-kb fragment. HNF-3a -/- ES cells...13. LacZ-specific primers were used to compare steady-state mRNA concentrations of (beta) -*galactosidase* in HNF-3a +/- and HNF-3a -/- EBs...

12/3,K/8 (Item 6 from file: 370)
DIALOG(R)File 370:Science

00504745 (USE 9 FOR FULLTEXT)
Quantitation of Transcription and Clonal Selection of Single Living Cells with (beta) -Lactamase as Reporter
Zlokarnik, Gregor; Negulescu, Paul A.; Knapp, Thomas E.; Mere, Lora; Burres, Neal; Feng, Luxin; Whitney, Michael; Roemer, Klaus; Tsien, Roger Y.
G. Zlokarnik, P. A. Negulescu, T. E. Knapp, L. Mere, N. Burres, L. Feng, M. Whitney, Aurora Biosciences, 11010 Torreyana Road, San Diego, CA 92121, USA. ; K. Roemer, University of the Saarland, D-66421 Homburg/Saar, Germany. ; R. Y. Tsien, Howard Hughes Medical Institute and Department of Pharmacology, University of California at San Diego, La Jolla, CA 92093-0647, USA.
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Language: English
Section Heading: Reports
Word Count: 3466

(THIS IS THE FULLTEXT)
(*980102*)
...Text: of gene expression in response to intrinsic developmental programs and extrinsic signals. To understand the regulation of gene expression, it is essential to use an *assay* of high sensitivity and fidelity that reports expression at the level of the single living cell. Such resolution is beneficial even in nominally homogeneous or clonal cell populations, because individual cells often prove quite heterogeneous in phenotype. A nondestructive *assay* compatible with high-throughput methods such as fluorescence-activated cell sorting (FACS) would enable powerful genetic *screens* for isolating individual variants with defined transcription responses. Correlation of gene expression with physiological responses and developmental fates would be facilitated by transcriptional *assays* that do not jeopardize cell survival. Current *assays* based on the reporter genes chloramphenicol acetyltransferase, secreted alkaline phosphatase, (beta) -*galactosidase*, and firefly luciferase (B1) typically require cell permeabilization or lack single-cell resolution, whereas the green fluorescent protein (GFP) is a relatively insensitive reporter due...

...Here we demonstrate the use of (beta) -lactamase and a membrane-permeant, fluorogenic substrate ester to *measure* gene expression in single live mammalian cells with high sensitivity and real-time response. (beta) -Lactamases are a family of bacterial enzymes that cleave

penicillins and cephalosporins very efficiently, in some cases at rates that approach the ultimate diffusion-controlled limit (B3). Because of their clinical importance in mediating *antibiotic* resistance, much is known about their three-dimensional structure, mechanism, optimal substrates, and inhibitors (B4) (B5) . A well-characterized isoform is the 29-kD, plasmid...

...of intensities at two or more wavelengths would then indicate the extent of enzyme-mediated cleavage with minimal interference from variations in cell size, probe *concentration*, excitation intensity, and emission sensitivity...

...substituent to lower the acid constant (pK.inf(a)) to 5.1 and make the fluorescence independent of pH values above 6. A glycine was *inserted* between the coumarin and the cephalosporin so that the fluorophore did not obstruct (beta) -lactamase attack. The coumarin was attached to the 7 position rather...

...is further quenched by its free thiol. The ratio of the emission intensity at 447 nm (blue) to that at 520 nm (green) is a *concentration* -independent *measure* of the extent of the reaction and increases 70-fold upon complete conversion. In phosphate-buffered saline (PBS) at pH 7.4, CCF2 is hydrolyzed...

...spontaneous hydrolysis rate of 1.2 (mid-dot) 10.sup(-7) s.sup(-1). Because of the >10.sup(8)-fold enzymatic acceleration, (beta) -lactamase *concentrations* down to 5 fM are readily quantifiable with CCF2 in vitro (Fig. 2B...incubated in aqueous buffer with 1 (mu) M CCF2/AM for 1 hour at room temperature, CCF2 accumulated to 50 to 100 (mu) M cytoplasmic *concentration*. CCF2 fluorescence in these cells was about 85 times that of cellular autofluorescence. Other cell lines loaded under these conditions were (listed with fluorescence relative...

...trimer of nuclear factor of activated T cells (NF-AT) binding sites (B16) (B17) expressed an average of 50 (beta) -lactamase molecules per cell as *measured* enzymatically in lysates. This very low amount of expression, resulting from leakiness of the NF-AT promoter, caused the CCF2-loaded cells to turn blue...

...loading (Fig. 3E). Such rapid conversion corresponded to 15,000 to 20,000 (beta) -lactamase molecules per cell. Thus, the sensitivity of the in situ *assay* could be adjusted simply by varying the time allowed for (beta) -lactamase action. The muscarinic antagonist pirenzepine prevented stimulation by carbachol (Fig. 3F). Adherent cells and nuclear receptors were equally amenable to this *assay*. For example, BHK (baby hamster kidney) cells, transfected with the reporter gene under the control of a promoter containing a pentamer of glucocorticoid response elements...

...gene expression, flow cytometry of the blue/green ratio in viable cells offers an automated selection procedure to complement or replace traditional selection based on *antibiotic* resistance (B19) . For example, Jurkat cells containing the NF-AT- (beta) -lactamase reporter, similar to C84, were transfected with the M.inf(1) receptor and a G418 resistance gene in the same pcDNA3 *vector*. After 3 weeks of selection in G418 medium, only about 40% of the cells were responsive to carbachol. Isolation of one of the blue cells...

...the C2 subclone (Fig. 3, C to F), in which ~80% of cells responded to carbachol, a percentage stable for more than 50 passages without *antibiotics*. Sorting can isolate even rarer blue or green cells. When (beta) -lactamase-expressing cells were artificially mixed with up to a 10.sup(6)-fold...

...of nonexpressing cells, a FACS was readily able to detect 50% and recover 30% of the positive cells for further culturing. Stained cells of either *color* were no different from unstained cells in subsequent viability and proliferation (B20The (beta) -lactamase *assay* also

revealed the dose-response and kinetic behavior of gene transcription at both the population and single-cell levels. We compared the average (beta) -lactamase content of C2 Jurkat cells (*measured* in cell lysates) with the percentages of blue, blue-green, and green cells *measured* by flow cytometry as a function of carbachol dose and as a function of time after stimulation with a maximal dose of carbachol (Fig. 4...

...blue cells expressing large amounts rather than a synchronized gradual increase in reporter content in all the cells. We could also watch the stimulus-evoked *color* change develop progressively in individual cells (Fig. 4C). Although all the cells had been subcloned from one highly responsive ancestor isolated by FACS, they showed...

...lactamase, which is important for its ability to track reductions in expression, by expressing the enzyme under the control of a constitutive viral promoter and *measuring* how rapidly the enzyme activity decayed after interruption of protein synthesis. The present (beta) -lactamase decayed with a half-life of 206 +/- 12 min (Fig...

...Transcriptional readouts are versatile *screens* for drug candidates, because most signaling pathways result in expression or repression of specific response elements and genes. Steps in disease progression likewise involve modulation...

...by transcriptional reporters even if the disease-causing mechanisms are not yet understood. The selectivity and sensitivity of the (beta) -lactamase transcriptional readout as a *screen* for drugs was assessed with a library of known pharmacophores (Microsource Discovery Systems, Gaylordsville, Connecticut). This library of 480 biologically active compounds was tested in a blind *screen* for muscarinic agonism and antagonism in 96-well microplates containing the C2 cell line. Known agonists (acetylcholine, arecholine, bethanechol, carbachol, methacholine, and pilocarpine) were identified at a test *concentration* of 5 (mu) M, and no other compounds in this set displayed agonist activity. In the primary *screen* for antagonist activity, 41 compounds were active (>80% inhibition of the response induced by 100 (mu) M carbachol). Rescreening in a cell line lacking the...

...A *concentration* of about 50 (beta) -lactamase molecules per cell, or 60 pM in a 1.4-pl Jurkat cell, produces in 16 hours a blue-green ratio well above that of nontransfected cells, as apparent by unaided *color* vision or *color* film (Fig. 3). By decreasing the duration of exposure to substrate, we *measured* expression levels up to about 20,000 molecules per cell in situ. A yet higher dynamic range was quantifiable in lysates (Fig. 2B). GFP is the only reporter of single-cell gene expression whose *assay* is less invasive than that of (beta) -lactamase, but because GFP is not catalytic, about 1 (mu) M cytosolic GFP (10.sup(5) to 10...hydrolysis of the substrate separates donor and acceptor. The donor then emits blue fluorescence whereas the acceptor is quenched. (C) Corrected emission spectra of equal *concentrations* of CCF2 (dashed green line) and its hydrolysis product (solid blue line) in PBS at pH 7.4 with excitation at 409 nm...

...Figure Removed

Figure F2
Caption: Hydrolysis of CCF2 by TEM-1 (beta) -lactamase. (A) Double-reciprocal plot of CCF2 molecules hydrolyzed per enzyme molecule per *second* (v) versus substrate *concentration* ([CCF2]). The rate of increase in blue fluorescence (460 nm, bandwidth 50 nm) with violet excitation light (395 nm, bandwidth 20 nm) was used as a *measure* of the rate of CCF2 hydrolysis. Seven substrate *concentrations* bracketing the K.inf(m) were prepared in PBS. Kinetics *measurements* were taken on a Cytofluor 4000 microtiter plate fluorimeter (Perseptive Biosystems) in quadruplicate with less than 5% substrate converted. The data were corrected for optical inner filtering of the solutions above substrate *concentrations* of 10 (mu) M. Error bars indicate standard errors. (B)

Linearity of the *assay* with enzyme *concentration*. Twofold serial dilutions of TEM-1 (beta) -lactamase in PBS were prepared in a 96-well clear-bottom plate. The rate of hydrolysis of CCF2...

...clone C2 stably transfected with M.inf(1) muscarinic receptor and NF-AT-(beta) -lactamase reporter genes. After 4 hours of incubation with the indicated *concentrations* of carbachol at 37.Deg.C, cells were collected, loaded with 1 (mu) M CCF2/AM for 1 hour at room temperature, and analyzed by...

...1 to 0.8. Matching samples of cells were lysed by three cycles of freeze-thawing in PBS. (beta) -Lactamase activity in the lysate was *measured* from the rate of hydrolysis of 1 nmol of CCF2 in 100 (mu) l in a Cytofluor 4000 microtiter plate fluorimeter (Perseptive Biosystems) (*). (B) Time

References and Notes:
...27. We thank J. Kadonaga and E. Raz for help with initial (beta) -lactamase *vectors*, S. Mobashery for purified TEM-1 enzyme, M. Simon for the M.inf(1) receptor gene, and S. Ho and G. Crabtree for a plasmid ...

12/3,K/9 (Item 7 from file: 370)
DIALOG(R)File 370:Science

00504733 (USE 9 FOR FULLTEXT)
Ultraviolet-Induced Cell Death Blocked by a Selenoprotein from a Human Dermatotropic Poxvirus
Shisler, Joanna L.; Senkevich, Tatiana G.; Berry, Marla J.; Moss, Bernard
J. L. Shisler, T. G. Senkevich, B. Moss, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 4 Center Drive, MSC 0445, Bethesda, MD 20892-0445, USA. ; M. J. Berry, Thyroid Division, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, Boston, MA 02115, USA.
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Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2330

(THIS IS THE FULLTEXT)
(*980102*)

...Text: Sec, within the region of homology with glutathione peroxidase (B4) . Recognition of the Sec codon is relatively inefficient and depends on a secondary structural selenocysteine *insertion* sequence (SECIS) element, which occurs immediately after UGA in prokaryotic mRNA and in the 3 (prime) noncoding region of eukaryotic mRNA (B11) . Inspection of the... virus is cytotoxic, we cloned the MC066L ORF and 3 (prime) noncoding sequence, with or without the HA epitope tag, into a nonviral eukaryotic expression *vector*. Transfected HeLa cells were incubated with .sup(75)Se, and lysates were analyzed by gel electrophoresis (B17) . Without the HA tag, a single labeled 30...

...controls (Fig. 2D, lane 4). With the epitope tag, a doublet was resolved (Fig. 2D, lane 3), indicating translation initiation at both the first and *second* in-frame AUG codons caused by nonoptimal sequences adjacent to the former. To evaluate a biological role of the MCV protein, we co-transfected cells with the MC066L *vector* (non-HA tag) and another plasmid that expresses the Escherichia coli lacZ gene. The pCI *vector* without the MC066L ORF or with the baculovirus P35 gene, a potent inhibitor of apoptosis (B18) , served as negative and positive controls, respectively. Transfected HeLa cells were irradiated with UV (Fig. 4 A) or treated with hydrogen peroxide (Fig. 4B) and, after further incubation, were stained for

(beta) -*galactosidase* ((beta) -Gal) and examined microscopically. Both UV irradiation and peroxide were found to induce cell rounding, membrane blebbing, and lifting of cells off the plate. Transfected cells were identified by their blue *color*, and the percentage that appeared flat and without signs of apoptosis was determined (B19) . The viability was higher when cells were transfected with MC066 or P35, compared to *vector* alone, indicating that the proteins encoded by these genes were protective. The ability of P35 to protect against UV-induced apoptosis was previously reported (B19...

...transfected genes were also obtained with a spontaneously immortalized human keratinocyte cell line, HaCaT (B21) , which undergoes apoptosis when UV-irradiated (B22) . Cells transfected with *vector* alone were more sensitive to UV irradiation (Fig. 4C) and hydrogen peroxide (Fig. 4D) than those expressing MC066L or P35. Unlike P35, MC066L did not...between MC066L ORF and the adjacent ORF; black bar, SECIS element within noncoding region. First ATG, translation initiation codon at start of HA epitope tag; *second* ATG, original initiation codon of MC066L ORF; TGA, alternative Sec or stop codon; TAG, stop codon at end of MC066L ORF; TCA, serine codon derived...

...labeled with .sup(75)Se. (D) SDS-PAGE autoradiograph of .sup(75)Se-labeled proteins. HeLa cells, untransfected (lane 1) or transfected with the pCI *vector* (lane 2), the pCI *vector* containing the epitope-tagged MC066L-A (lane 3), or the untagged MC066L with 3 (prime) -noncoding sequences (lane 4), were labeled with .sup(75)Se...

...control plasmid that expresses human growth hormone, into human embryonic kidney 293 cells. Deiodinase activity, normalized to units of human growth hormone (B16) , of duplicate *assays* from two independent transfection experiments is shown...

...peroxide-induced cell death. HeLa (A, B, E, and F) or HaCaT (C and D) cells were co-transfected with CMV- (beta) -Gal and pCI *vector*, pCI-P35, or pCI-MC066L-A. Thirty hours after transfection, the cells were UV irradiated (A and C) or were treated with hydrogen peroxide (B...

References and Notes:

...nucleotides 661 to 932 or 661 to 797 (Fig. 1) as subcloned downstream of the rat type 1 deiodinase coding region in the mammalian expression *vector* pUHD10 [M. Gossen and H. Bujard, Proc. Natl. Acad. Sci. U.S.A. 89, 5547 (1992)]. Plasmids containing the wild-type deiodinase SECIS element or

12/3,K/10 (Item 8 from file: 370)

00504540 (USE 9 FOR FULLTEXT)

Specific Inhibition of Stat3 Signal Transduction by PIAS3

Chung, Chan D.; Liao, Jiayu; Liu, Bin; Rao, Xiaoping; Jay, Philippe; Berta, Philippe; Shuai, Ke

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Science Vol. 278 5344 pp. 1803
Publication Date: 12-05-1997 (*971205*) Publication Year: 1997
Document Type: Journal ISSN: 0036-8075
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Word Count: 1944

(THIS IS THE FULLTEXT)
(*971205*)

...Text: We cloned a protein named PIAS1, which can specifically interact with Stat1 (another member of the STAT family), by the yeast two-hybrid *assays* (B6) . We searched the expressed sequence tag (EST) database for other PIAS family members and identified a human EST clone encoding a polypeptide related to the COOH-terminal portion of PIAS1 (B7) . We obtained a full-length cDNA containing an open reading frame of 583 amino acids by *screening* a mouse thymus library with the human EST clone (B8) . The corresponding protein, named PIAS3, contains a putative zinc-binding motif [C.inf(2)-(X...

...tested the effect of PIAS3 on the DNA-binding activity of Stat3. Nuclear extracts from HepG2 cells were prepared and analyzed in mobility gel shift *assays*, with a high-affinity Stat3-binding site as the probe (B1) (B11) . Treatment with IL-6 induced the binding of three distinct gel shift complexesTo test the effect of PIAS3 on Stat3-mediated gene activation, we transiently transfected HepG2 cells with expression *vectors* encoding Stat3 and FLAG-tagged PIAS3. Interleukin-6 can induce the association of PIAS3 with Stat3 in HepG2 cells (B10) . A luciferase reporter construct [(4 ...
...g and 1 (mu) g), Stat3-mediated induction of luciferase expression in response to IL-6 stimulation was inhibited (Fig. 4A). We also performed luciferase *assays* in human embryonic 293 cells. Interferon a (IFN-a) stimulation can activate Stat3 in 293 cells (B10) (B12) . Cells cotransfected with Stat3 and (4 x...

...activity of JAKs. The identification of a PIAS protein that can directly inhibit STAT function indicates that JAK-STAT signaling pathways can be suppressed at *multiple* steps, in a general or specific manner. It seems that the overall strength of STAT signaling for a given cell type may be largely affected...

...Figure Removed

Figure F3
Caption: Inhibition of the DNA-binding activity of Stat3 by PIAS3. (A) Electrophoretic mobility shift *assays* were performed with nuclear extracts prepared from HepG2 cells with (+) or without (-) IL-6 treatment in the absence or presence of various amounts of either GST or GST-PIAS3 proteins (20 to 200 ng) as indicated. Mobility shift *assays* were done as described (B14) . The probe used is a high-affinity Stat3-binding site to which both Stat1 and Stat3 can bind (B1) (B15...the NF- (kappa) B-binding site in the promoter of the NF- (kappa) B inhibitor I- (kappa) B gene (B15) . GST-PIAS3 was constructed by *insertion* of the cDNA into the Sal I and Not I cloning sites of pGEX4T-1 (Pharmacia). The *concentration* of GST-PIAS3 was estimated on 7% SDS-polyacrylamide gel electrophoresis with various dilutions of bovine serum albumin as the standard...

...gene activation in response to IL-6. HepG2 cells were transiently transfected with (4 x)IRF-1 luciferase reporter construct together with an empty expression *vector*, Stat3, or various amounts of FLAG-PIAS3 *vectors*, alone or in combination as indicated. Twenty-four hours after transfection, cultures were either left untreated (open columns) or treated with IL-6 (10 ng/ml) (R&D Systems, Minneapolis, Minnesota) for 6 hours (solid columns), and cell extracts were prepared and *measured* for luciferase activity (Promega, Madison, Wisconsin). (B) Inhibition of Stat3-mediated gene activation in response to IFN-a. Human 293 cells were transfected with (4...

...activity was determined. (C) The effect of PIAS3 on Stat1-mediated gene activation. Same as (B), except that Stat3 was replaced with Stat1 in cotransfection *assays*. FLAG-PIAS3 was constructed by *insertion* of the cDNA into the Sal I and Hind III sites of pCMV5-FLAG. HepG2 cells were

transfected by a modified calcium phosphate method (B16...
...shown are taken from one representative experiment and were repeated at least three times. The relative luciferase units were corrected for relative expression of (beta) -*galactosidase*.

12/3,K/11 (Item 9 from file: 370)
DIALOG(R)File 370:Science

00501668 (USE 9 FOR FULLTEXT)
Binding of Neuroligins to PSD-95
Irie, M.; Hata, Y.; Takeuchi, M.; Ichtchenko, K.; Toyoda, A.; Hirao, K.; Takai, Y.; Rosahl, T. W.; Suedhof, T. C.;
M. Irie, M. Takeuchi, A. Toyoda, K. Hirao, Takai Biotimer Project, ERATO, Japan Science and Technology Corporation, 2-2-10, Murotani, Nishi-ku, Kobe, 651-22, Japan. Y. Hata, Howard Hughes Medical Institute and Department of Molecular Genetics, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235-9050, USA, and Takai Biotimer Project, ERATO, Japan Science and Technology Corporation, 2-2-10, Murotani, Nishi-ku, Kobe 651-22, Japan. ; K. Ichtchenko, T. W. Rosahl, T. C. Suedhof, Howard Hughes Medical Institute and Department of Molecular Genetics, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75235-9050, USA. ; Y. Takai, Takai Biotimer Project ERATO, Japan Science and Technology Corporation, 2-2-10, Murotani, Nishi-ku, Kobe 651-22, Japan, and Department of Molecular Biology and Biochemistry, Osaka University Medical School, Suita 565, Japan.
Science Vol. 277 5331 pp. 1511
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Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 3396

(THIS IS THE FULLTEXT)
(*970905*)

...Abstract: form intercellular junctions. Neuroligins bind to the third PDZ domain of PSD-95, whereas NMDA2 receptors and K.sup(+) channels interact with the first and *second* PDZ domains. Thus different PDZ domains of PSD-95 are specialized for distinct functions. PSD-95 may recruit ion channels and neurotransmitter receptors to intercellular...

...Text: and (beta) -neurexins localized on separate cells bind to each other and form a heterotypic intercellular junction regulated by alternative splicing (B6) . We quantified the *concentrations* of neurexin and neuroligin mRNAs in mouse brain during postnatal development to determine their developmental profile (B7) . We used total RNA prepared from forebrain and...

...increase in the amounts of neurexin and neuroligin mRNAs with peak expression between postnatal days 5 and 10. Thereafter, the mRNAs declined to a constant *concentration* of 25 to 40% of the peak quantities (not shown). By comparison, analysis of expression of GAP-43, a protein implicated in axonal pathfinding, also...

...intracellular anchoring mechanism for this junction, we have searched for binding proteins for the cytoplasmic domain of neuroligins. We used yeast two-hybrid selection and *screened* a prey cDNA library from rat brain with a bait plasmid encoding the cytoplasmic tail of neuroligin 2 (B8) . Sixteen isolates were obtained, two of...

...other neuroligins also interact with PSD-95, and whether isoforms of PSD-95 also bind to neuroligins, we quantified the yeast two-hybrid interactions by *measuring* (beta) -*galactosidase* transactivation (Table 1). For this purpose, isoforms of PSD-95 were cloned first by polymerase

chain reaction (PCR) and then by standard cDNA cloning techniques; then they were *inserted* into the appropriate yeast two-hybrid *vectors* (B9) . The cytoplasmic domains of all three neuroligins interacted with the NH.inf(2)-terminus of PSD-95, which contains the three PDZ domains. Furthermore...

...The first and *second*, but not the third, PDZ domains of PSD-95, bind tightly to the cytoplasmic COOH-termini of NMDA receptors and K.sup(+) channels, which suggests that the three PDZ domains of PSD-95 are not equivalent (B3) . We therefore used yeast two-hybrid *assays* to investigate the ability of individual PDZ domains from PSD-95 to bind to neuroligins, K.sup(+) channels, and NMDA receptors (Table 1). The first and *second* PDZ domains alone bound only weakly to the cytoplasmic tail of neuroligins; in combination, binding was much stronger. These two PDZ domains, however, interacted strongly...the NMDA2 receptor and K.sup(+) channels (Thr/Ser-Xaa-Val, where Xaa is any amino acid) is required for binding to the first and *second* PDZ domains of PSD-95 (B3) . As revealed by the crystal structure (B10) , the third PDZ domain of PSD-95 also binds a COOH-terminal...
...may bind to the third PDZ domain of PSD-95 via their COOH-terminus. We tested this hypothesis with deletion constructs in yeast two-hybrid *assays* (Fig. 1). The COOH-terminal 48 residues from neuroligin 1 were sufficient for binding to PSD-95. Deletion of only three COOH-terminal amino acids...

...Because yeast two-hybrid *assays* are prone to artifacts and do not allow an assessment of binding affinities, we analyzed the binding of neuroligins to PSD-95 directly by surface...

...lacking the last three amino acids (NL1-2, negative control), a peptide containing the COOH-terminal 16 residues of the NMDA2 receptor (NMDAR2A), and a *second* negative control with a 17-residue peptide that also ends with a Thr-Xaa-Val motif from the interior sequence of PSD-95. Superfusion of...

...domains resulted in a strong signal for the NMDA receptor and the wild-type neuroligin peptides but not for the truncated neuroligin peptide or the *second* control peptide (Fig. 2A). Superfusion with a GST fusion protein that contained the COOH-terminal domains of PSD-95 (SH3 and guanylate kinase domains) failed...
...Xaa-Val sequence because the control peptide from the interior PSD-95 sequence did not interact. Analysis of the binding affinity with a series of *concentrations* of GST fusion protein allowed us to estimate the affinity under our *assay* conditions (K.inf(M) approximate equal to 2.3 +/- 0.4 x 10.sup(-7) M for neuroligin 1; K.inf(M) approximate equal to ...

...was produced as a fusion protein with the COOH-terminus of immunoglobulin G (IgG), either without (1 (beta) .sup(-)) or with (1 (beta) .sup(+)) an *insert* in splice site 4, the splice site that regulates neuroligin binding (B4) . We then immobilized the recombinant neurexins and a control IgG protein and performed *second* bridging protein in brain mediates the interaction of PSD-95 with neuroligins or neurexin 1 (beta) .sup(-). To rule out these possibilities, we applied proteins...

...conclusion is supported by the following findings: (i) All three neuroligins specifically interact with three members of the PSD-95 family in yeast two-hybrid *assays*. (ii) The interactions require the COOH-terminal three amino acids of neuroligins. (iii) These interactions are mediated by the third PDZ domain, which does not ... Begin Table : Columns 1 - 6 of 9

Caption:
Interactions between the cytoplasmic domains of neuroligins and various PDZ domains. Data list (beta) -*galactosidase* activities of yeast strains harboring the respective bait and prey plasmids. Single colonies

from yeast cotransformed with the listed prey and bait *vectors* were selected on plates supplemented with minimal medium that lacked uracil, tryptophan, and leucine and were grown in liquid culture in the presence of selection medium for 40 hours. (beta) -*Galactosidase* activity and protein concentrations of cell extracts were determined in triplicate Reference B8 . Data shown are nanomoles of substrate hydrolyzed per minute per milligram of protein +/- SD after background subtraction; -, not tested; <20, no detectable activity. For plasmids, see Reference B9 .

Prey *vector*	Bait *vector* (in pBT			
	NL1-1	NL1-10	NL2-1	NL3
PSD-95				
pVP16PSD95-2 (PDZ 1-3)	1437 +/- 24	453 +/- 10	2635 +/- 48	1350 +/- 6
pVP16SAP90...				

...9

Begin Table : Columns 7 - 9 of 9

Caption:
Interactions between the cytoplasmic domains of neuroligins and various PDZ domains. Data list (beta) -*galactosidase* activities of yeast strains harboring the respective bait and prey plasmids. Single colonies from yeast cotransformed with the listed prey and bait *vectors* were selected on plates supplemented with minimal medium that lacked uracil, tryptophan, and leucine and were grown in liquid culture in the presence of selection medium for 40 hours. (beta) -*Galactosidase* activity and protein concentrations of cell extracts were determined in triplicate Reference B8 . Data shown are nanomoles of substrate hydrolyzed per minute per milligram of protein +/- SD after background subtraction; -, not tested; <20, no detectable activity. For plasmids, see Reference B9 .

Prey *vector*	NMDAR2A	NMDAR2B	Shaker
PSD-95			
pVP16PSD95-2	3303 +/- 159	2875 +/- 113	2481 +/- 263
pVP16SAP90-5	2460 +/- 33	-	328 +/- 30
pVP16SAP90-6	639 +/- 23	-	256 +/- 3...

...F1

Caption: Sequence dependence of neuroligin interaction with PSD-95. Parts of cytoplasmic tails of neuroligins that were deleted are indicated as hatched regions. (beta) -*Galactosidase* activities are given in nanomoles per minute per milligram of protein (B8) (B9...

...COOH-terminal IgG domain fused to the following NH.inf(2)-terminal sequences were used: IgG-neurexin 1 (beta) .sup(+), neurexin 1 (beta) with an *insert* in splice site 4; IgG-neurexin 1 (beta) .sup(-), neurexin 1 (beta) without an *insert* at splice site 4; IgG-control, a short NH.inf(2)-terminal control sequence. Affinity chromatography fractions obtained with immobilized IgG fusion proteins were blotted...

...independent monoclonal antibodies to PSD-95, one of which was obtained from M. B. Kennedy (not shown). Strong reactivity with the PSD-95 antibody of *multiple* low bands in the origin and flow-through lanes (right) is caused by rapid proteolysis of PSD-95 in brain homogenates after solubilization; these bands...

References and Notes:

...8. A rat brain cDNA library in the yeast-two hybrid prey *vector* pVP16 [A. B. Vojtek, S. M. Hollenberg, J. A. Cooper, Cell 74, 205 (1993)] was screened as described [S. Fields and O. Song, Nature 340, 245 (1994)] (B14) with the bait *vector* pBTM116-NL2-1 (B8) . Of 16 prey clones

isolated, pPrey500 encodes residues 1 to 287, and pPrey514 encodes residues 308 to 425 of PSD-95 (B1) . (beta) -*Galactosidase* assays were corrected for protein concentration [M. D. Rose, F. Winston, P. Hieter, Methods in Yeast Genetics (Cold Spring Harbor Laboratory Press, Cold Spring Harbor...SAP102. pDLG70 corresponds to PSD-93/chapsyn with several amino acid differences that may be due to alternative splicing or sequencing variations. The neuroligin bait *vectors* used encode the following residues of the rat proteins (B4) : pBTM116-NL1-1, 718-843; pBTM116-NL1-2, 718-840; pBTM116-NL1-5, 718-828...

...of the rat NMDA-2A and -2B receptors. pBTM116Shaker encodes residues 645-654 of the rabbit Kv1.4 K.sup(+) channel isoform. The different prey *vectors* are from rat except for ZO-1 (mouse) and dlg-A (Drosophila) and encode the following residues: PSD-95, pVP16PSD-95-2 = 1-431; pVP16SAP90 ...

...12. IgG fusion proteins of the extracellular domains of neurexin 1 (beta) with and without an *insert* in splice site 4 and control IgG fusion protein were purified from transfected COS cells (B4) . Twelve rat brains were homogenized in 48 ml of...

12/3,K/12 (Item 10 from file: 370)

00501578 (USE 9 FOR FULLTEXT)

A Cytoplasmic Inhibitor of the JNK Signal Transduction Pathway

Dickens, Martin; Rogers, Jeffrey S.; Cavanagh, Julie; Raitano, Art; Xia, Zhengui; Halpern, Jocelyn R.; Greenberg, Michael E.; Sawyers, Charles L.; Davis, Roger J.
M. Dickens, J. S. Rogers, J. Cavanagh, R. J. Davis, Howard Hughes Medical Institute and Program in Molecular Medicine, Department of Biochemistry and Molecular Biology, University of Massachusetts Medical School, 373 Plantation Street, Worcester, MA 01605, USA. ; A. Raitano, J. R. Halpern, C. L. Sawyers, Department of Medicine, University of California School of Medicine, Los Angeles, CA 90095, USA. ; Z. Xia and M. E. Greenberg, Division of Neuroscience, Department of Neurology, Children's Hospital, and Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA.

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Publication Date: 8-01-1997 (*970801*) Publication Year: 1997
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2461

(THIS IS THE FULLTEXT)
(*970801*)

...Text: proteins is one mechanism that may account for the retention of JNK in specific regions of the cell. Anchor proteins participate in the regulation of *multiple* signal transduction pathways, including the nuclear factor kappa B inhibitor I (kappa) B, the A-kinase anchor protein (AKAP) group of proteins that bind type...

...We examined the interaction of JIP-1 and JNK1 by coimmunoprecipitation analysis of lysates from COS-1 cells that were transfected with *vectors* encoding JIP-1 and JNK (Fig. 1B). JIP-1 was detected in JNK1 immunoprecipitates by protein immunoblot analysis. Conversely, JNK1 was detected in JIP-1...

...To test whether JIP-1 interacts directly with JNK, we performed in vitro binding *assays*. The putative JBD (residues 127 to 281) was expressed as a glutathione-S-transferase (GST) fusion protein. Recombinant JNK prepared by in vitro translation (B14) was incubated with GST-JIP-1 that was immobilized on glutathione-agarose. A similar amount of binding to JIP-1 was detected in *assays* with 10 different human JNK isoforms (B14...an antibody to Flag (top panel). (C) Deletion analysis of JIP-1. The GST

fusion proteins (Coomassie-stained gel) and the results of the binding *assay* (immunoblot) are shown. The binding of JNK1 and JNK2 to GST and GST fusion proteins corresponding to residues 127 to 202, 203 to 281, 164...

...127 to 281) bound to glutathione-Sepharose, and bound proteins were detected by protein immunoblot analysis with an antibody to Flag. The effect of increasing *concentrations* (0, 4, 8, 16, 32, and 64 (mu) g/ml) of synthetic peptide corresponding to JIP-1 residues 148 to 174 or to a peptide...

...without mouse interleukin-1 (IL-1) (10 ng/ml) (JNK and p38) or 100 nM phorbol 12-myristate 13-acetate (ERK). Protein kinase activity was *measured* in immune complex kinase *assays* by means of 3 (mu) g of substrate. ERK, JNK, and p38 MAP kinase were *assayed* with c-Jun, ATF2, and myelin basic protein as substrates, respectively. The effect of the addition of 3 (mu) g of GST or GST-JIP...inhibits pre-B cell transformation by Bcr-Abl. (A) JNK activation by v-Abl and Bcr-Abl is suppressed by JIP-1. JNK activity was *measured* in an immune-complex kinase *assay* of 293 cell lysates by means of a polyclonal JNK antibody and the substrate GST-Jun. The effect of transfection of the cells with plasmid *vectors* that express v-Abl, Bcr-Abl, and the JBD of JIP-1 (residues 127 to 281) is presented. (B) Primary mouse marrow cells were infected...

...10 is presented. The data shown are derived from three independent experiments plated in triplicate. The empty box in the first line indicates that the *vector* does not contain an *insert* in this position. (C) Photomicrographs of representative plates from (B) are shown...

References and Notes:
...cDNA library in the yeast strain L40 [Z. Galcheva-Gargova et al., Science 272, 1797 (1996)]. The bait plasmid (pLexA-JNK1) was constructed by the *insertion* of JNK1 in the polylinker of pBTM116. Full-length JIP-1 clones were obtained from a mouse brain (lambda) ZAPII cDNA library (Stratagene), and expression *vectors* were constructed with the *vectors* pCMV5, pCDNA3 (Invitrogen), and pGEX-3X (Pharmacia LKB Biotechnology). Mutations were constructed with the polymerase chain reaction. The sequence of the JIP-1 cDNA has...

...19. Luciferase reporter assays in CHO cells were performed 48 hours after transfection with the use of (beta) -*galactosidase* ((beta) -Gal) to measure transfection efficiency (B14) . The cells were activated by treatment with fetal calf serum (10%). The data are presented as the relative...

12/3,K/13 (Item 11 from file: 370)
DIALOG(R)File 370:Science

00501249 (USE 9 FOR FULLTEXT)
Coordination of Drosophila Metamorphosis by Two Ecdysone-Induced Nuclear Receptors
White, Kevin P.; Hurban, Patrick; Watanabe, Toshiki; Hogness, David S.
Departments of Developmental Biology and Biochemistry, Beckman Center B300, Stanford University School of Medicine, Stanford, CA 94305-5427, USA.
Science Vol. 276 5309 pp. 114
Publication Date: 4-04-1997 (*970404*) Publication Year: 1997
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 3697

(THIS IS THE FULLTEXT)
(*970404*)

...Text: histolysis of larval tissues (B2) . Our interest is in the

ecdysone-activated genetic regulatory hierarchies that underlie these two sets of responses. Here we identify *multiple* functions for ecdysone-inducible nuclear receptors that coordinate these two distinct hierarchies...
...expression is induced as a primary response to ecdysone, and they are subsequently repressed by proteins encoded by ecdysone-induced genes. E74 and E75 encode *multiple* isoforms from overlapping, differently regulated transcription units, of which E74A and E75A exhibit these early gene properties (Fig. 1A...

...isoform was used in these experiments, it is probable that the other two isoforms (EcR-A and EcR-B2) would interact with DHR3 in this *assay* because the NH.inf(2)-terminally truncated (Delta) 361 EcR (Fig. 2A) also interacts with DHR3 (Fig. 2B). (Delta) 361 EcR lacks both the DBD...105 DHR3 also repress this ecdysone inducibility (Fig. 2C), which indicates that the DHR3 sequences sufficient for interaction with EcR in the yeast two-hybrid *assay* are also sufficient for this repression. To test whether DHR3 can inhibit the ecdysone induction of the E74A and E75A early genes in the animal...

...The *second* function of DHR3 provides a temporal linkage between the two ecdysone responses by activating (beta) FTZF1. One indication of this activation is the overlap in...

...proteins-an overlap that includes the peak of (beta) FTZF1 mRNA expression (B15) (arrows at 6 to 8 hours after pupariation in Fig. 1B). A *second* indication is the observation that the strongest binding sites of DHR3 protein to polytene chromosomes include the 75D locus, which corresponds to the (beta) FTZF1...single consensus DHR3 sites at each of these loci (B20) . To determine if DHR3 can associate with E75B on these elements, we performed gel shift *assays* using DNA sequences containing one or the other of these high-affinity sites. Results were similar for both sites, and gel shifts using the stronger...Figure Removed

Figure F2
Caption: DHR3 interacts with EcR in a two-hybrid *assay*, inhibits transactivation by the ecdysone receptor in tissue culture, and represses early gene induction in vivo. (A) Schematic of the EcR and DHR3 nuclear receptors...

...GAL4 fusions to these nuclear receptors were constructed with either the GAL4 DBD or the GAL4 activation domain (AD). EcR, DHR3, and their derivatives were *inserted* into pAS2 or pGAD-GH (B22) . Fusion proteins were expressed in the yeast strain YRG-2, and the resulting strains were tested for growth and...

...fusion proteins. Quantitation of (beta) -Gal activity reveals that the EcR/DHR3 interaction is of the same magnitude as the EcR/USP interaction in this *assay*. (C) DHR3 can repress transactivation by EcR in tissue culture. S2 tissue culture cells, which contain endogenous EcR/USP, were cotransfected with an ecdysone-inducible...

...x 10.sup(-6) M ecdysone and 80 mM CuSO.inf(4) for 24 hours. Ecdysone induction of the 7 x EcRE:LacZ transgene was *measured* by the o-nitropheynl (beta) -o-galactopyranoside (ONPG) *assay* for (beta) -Gal activity. Fold repression by DHR3, (Delta) 83 DHR3, or (Delta) 105 DHR3 was determined by dividing the fold induction by ecdysone alone...

...83 DHR3) with the use of a yw strain as the progenitor. Larvae were reared on media containing bromophenol blue to assist in staging by *color* of the gut, as described (B25) . Mid-third instar blue-gut larvae carrying these transgenes were collected and either held at 22.Deg.C or...

...these, the 34 sites listed in this table as binding to the X chromosome and to the left (L) and right (R) arms of the *second* and third chromosomes stained most strongly. Sites corresponding to the DHR3, E74, E75, and FTZF1 genes are labled; asterisks indicate other puff sites.

X 2L...

References and Notes:
...23. Yeast strains were constructed by transforming the strain YRG-2 (Stratagene) with the indicated open reading frames *inserted* into the *vectors* pGAD-GH and pAS2, which respectively contain Leu or Trp selectable markers. Strains were assayed for growth on synthetic dropout complete (SDC)-leu/trp/his plates containing 50 mM 3-aminotriazole. (beta) -*Galactosidase* ((beta) -Gal) assays were performed with ONPG. Each strain was assayed by protein immunoblot for expression of the appropriate fusion proteins...26. Baculovirus reagents were obtained from Pharmingen. The DHR3, E75B, and USP open reading frames were *inserted* into the baculovirus transfer *vector* pVL1393; DHR3 was also *inserted* into pAcGHLT to produce GST-DHR3, and the empty *vector* was used to produce GST alone. Crude extracts for chromatography were prepared by harvesting infected cells through brief centrifugation and resuspension in bv150 [150 mM...

12/3,K/14 (Item 12 from file: 370)
DIALOG(R)File 370:Science

00501166 (USE 9 FOR FULLTEXT)
Combinatorial Control Required for the Specificity of Yeast MAPK Signaling
Madhani, Hiten D.; Fink, Gerald R.
Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA.
Science Vol. 275 5304 pp. 1314
Publication Date: 2-28-1997 (*970228*) Publication Year: 1997
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2321

(THIS IS THE FULLTEXT)
(*970228*)

...Text: in yeast requires combinatorial control. Ste12 can act as a homomultimer to promote pheromone-responsive transcription (B7) . During filamentation and invasion, Ste12 acts with a *second* transcription factor, Tec1, to drive transcription that is specifically responsive to the MAPK pathway that promotes filamentation and invasion. The TEC1 FRE is necessary for...
...specific gene expression. (A) Expression of FRE(Ty1)::lacZ in haploid a cells. (beta) -Gal activities (in nanomoles per minute per milligram of protein) were *measured* on three transformants (B13) . The relevant genotypes are indicated at the bottom of the graph. Strains used in this study were of the (Sigma) 1278b background (B23) . PRE mutant and TCS mutant indicate reporter mutants *assayed* in the wild-type (WT) haploid strain. Error bars indicate standard deviations. (B) Expression of FRE(Ty1)::lacZ in a/a diploid cells. Expression was *measured* in homozygous diploids of the indicated genotypes that carried either a *vector* plasmid or a plasmid encoding the dominant activated allele STE11-4 (B13...

...development. (A) Expression of TEC1 promoter mutants in wild-type diploid cells. Expression of TEC1::lacZ promoter fusions carrying point mutations in the FRE was *measured* on three transformants (B17) . Solid bars show the average activity in nanomoles per minute per milligram of protein, and error bars show the standard deviation...

...leu2::hisG/leu2::hisG) containing a wild-type TEC1 allele integrated at URA3 (wild type) was compared to that of cells transformed with the empty *vector* (null) or tec1 alleles containing mutations in the FRE. The strains contained a plasmid encoding STE11-4 on a LEU2, CEN *vector* (right

column) or a LEU2, CEN control *vector* that lacked an *insert* (left column). Plates were incubated for 5 days at 30.Deg.C...

...shown. B, bound; F, free. .sup(32)P-labeled Ty1 FRE was applied to the gel after incubation with the indicated components (B20) . Approximate protein *concentrations* were as follows: MBP, 5 x 10.sup(-9) M; MBP-Tec1, 6 x 10.sup(-10) M; and MBP-Ste12, 6 x 10.sup(-10) M. .sup(32)P-labeled Ty1 FRE *concentration* was approximately 3 x 10.sup(-10) M. Unlabeled competitor DNAs were added as indicated and are labeled as follows: TY1, wild-type Ty1 FRE...

References and Notes:
...the Ty1 FRE (CATTCTTCTGTTTTGGAAGCTGAAACG) flanked by Xho I ends. The FRE extends from positions +60 to +86 with respect to the Ty1 (delta) sequence. (beta) -*galactosidase* ((beta) -Gal) assays were performed as described (B8) on extracts of exponentially growing cells in liquid synthetic complete medium lacking uracil for haploid cells and...primer whose 5 (prime) end lies 55 bp upstream of the 5 (prime) end of the FREs present in the FRE::lacZ constructs, and a *second* primer that lies 181 bp downstream of the FRE *inserts*. Protein samples were incubated with the probe for 10 min in binding buffer in a 50- (mu) l volume. Approximate concentrations were as follows: probe... °

12/3,K/15 (Item 13 from file: 370)
DIALOG(R)File 370:Science

00500986 (USE 9 FOR FULLTEXT)
Global Climate and Infectious Disease: The Cholera Paradigm.sup()
Colwell, Rita R.;
The author is in the University of Maryland Biotechnology Institute, 4321 Hartwick Road, Suite 550,College Park, MD 20740, USA.
Science Vol. 274 5295 pp. 2025
Publication Date: 12-20-1996 (*961220*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Policies
Word Count: 7430

(THIS IS THE FULLTEXT)
(*961220*)

...Text: Control. Leptospirosis is a bacterial infection transmitted by animal urine or excrement that seeps into food and water supplies. The disease can be treated with *antibiotics*, and its spread can be curtailed by methods similar to those used for cholera epidemics...The *second* pandemic (1829 to 1851) is believed to have begun in Russia, where citizens of Moscow were particularly hard hit. The pandemic spread across the Atlantic...

...The *second* pandemic reappeared in a region of London, close to where Dr. John Snow, physician to Queen Victoria, lived. In the summer of 1849, John Snow...September. By then, the epidemic had begun to wane, but this action probably represents the first instance on record of the implementation of an appropriate *measure* to prevent the transmission of a waterborne disease (B10) . Thus, John Snow is given credit for both stopping the epidemic and proving its connection to...

...lipopolysaccharide virulence determinants (B15) . Furthermore, there is a deletion of about 22 kb of DNA from the 01 chromosome in the rfb region and an *insertion* of a new 35-kb region of DNA that specifies the 0139 LPS and capsules (B16) . The occurrence of epidemics caused by V. cholerae 0139 ...different sugar composition (B17) . The evidence further suggests the V. cholerae 01 El Tor gave rise to 0139 by acquisition of novel DNA which was *inserted* into, and replaced part of, the O antigen gene cluster of the recipient strain. From the sequence of the novel DNA, two open reading

frames present in high *concentrations* (B25) (B26) (B27) , that is, high *concentrations* of organic nutrients can compensate to a degree for lack of salt. Similarly in fresh water, the presence of divalent cations can compensate for Na...For a bacterium capable of attachment to, and colonization of surfaces, surface specificity often is critical. V. cholerae, however, offers *multiple* recognition sites, including not only the intestinal mucosa and brush border cells of the mammalian gut, but also the hindgut mucosa of blue crabs, which...water supplies. Filtering water at the time of collection and just before drinking is a successful means of removing cyclops, a planktonic crustacean copepod and *vector* of the guinea worm, which causes dracunculiasis. The crustacean cyclops-associated worm is removed by filtration with polyester cloth and is now a recommended method...

12/3,K/16 (Item 14 from file: 370)
DIALOG(R)File 370:Science

00500975 (USE 9 FOR FULLTEXT)
NF-AT-Driven Interleukin-4 Transcription Potentiated by NIP45
Hodge, Martin R.; Chun, Hyung J.; Rengarajan, Jyothi; Alt, Aya; Lieberson, Rebecca; Glimcher, Laurie H.
Department of Cancer Biology, Harvard School of Public Health and Department of Medicine, Harvard Medical School, Boston, MA 02115, USA.
Science Vol. 274 5295 pp. 1903
Publication Date: 12-13-1996 (*961213*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2627

(THIS IS THE FULLTEXT)
(*961213*)

...Text: tissue-specific IL-4 expression (B3) . c-Maf acts in synergy with NF-AT proteins to transactivate the IL-4 promoter. The inducible expression of *multiple* cytokine genes and cell surface proteins after T cell receptor stimulation requires members of the NF-AT transcription factor family (B1) (B4) (B5) (B6...
...B10) . One class of interactors encoding a fusion protein with apparently high affinity for the NF-ATp(RHD)-Gal4 bait, as exhibited by high (beta) -*galactosidase* activity and ability to confer leucine prototrophy, was isolated. The interaction with this factor was specific, because no interaction was detected with baits that encoded...

...The ability of this polypeptide to interact specifically with NF-ATp in vivo was tested in mammalian cells. The 1.9-kb *insert* was subcloned into a mammalian expression *vector* that fused the coding region to an epitope tag from an influenza hemagglutinin (HA) peptide. This tagged construct was cotransfected with an NF-ATp expression...

...Because the transcript size detected by northern blot analysis (below) was approximately 3.1 kb, the 1.9-kb *insert* from this clone was used to *screen* a T cell cDNA library to identify full-length clones. *Screening* of a library containing approximately 8 x 10.sup(5) clones yielded an isolate that contained an additional segment of 180 bp located 868 bp...did not affect the subcellular localization of this NIP45 (B11) . Nuclear trafficking of NF-AT4 in HepG2 cells in response to changes in intracellular calcium *concentrations* was not affected by the overexpression of exogenous NIP45 (B11...
...transactivates NF-AT-driven transcription in this cell line in the absence of exogenous stimulation (B12) . The cDNA encoding NIP45 was cloned into an expression *vector* and cotransfected into HepG2 cells with NF-ATp and a reporter gene containing tandem copies of the NF-AT binding site (3XNF-AT-CAT). Transfection...

...NIP45 cannot act on its own to transactivate an NF-AT target sequence. Overexpression of NF-ATp alone resulted in substantial (six times greater than *vector* control) transactivation of the NF-AT-CAT reporter (Fig. 4A) (B12) . Cotransfection of NIP45 plus NF-ATp resulted in a four to five times increase in CAT activity relative to transfection with NF-ATp alone and a 25 to 30 times increase over that seen with *vector* alone (Fig. 4A). This increase was not observed when a mutant 3XNF-AT-CAT reporter or a control major histocompatability complex (MHC) class II promoter...B lymphoma cells stably transfected with NF-ATp and c-maf expression *vectors* produce low amounts of endogencus IL-4 protein (B3) . In contrast, endogenous IL-4 is not detected upon transient transfection, which presumably reflects the lower...

...to levels detectable in a transient expression system. Therefore, M12 cells were transiently cotransfected with c-maf and NF-ATp and either NIP45 or control *vector*. Four independent sets of transient transfections were done and *assayed* for secretion of IL-4 into the culture supernatant. For each set of transfections, inclusion of NIP45 led to a 50 to 200 times increase...

...Interacting Protein (NIP45). (A) Interaction of NIP45 and NF-ATp in HepG2 cells: cDNA from the NIP45 plasmid was cloned into the epitope tag expression *vector* pCEP4-HA (B16) . This construct results in the in-frame fusion of amino acids with the sequence YPYDVPDYA (B17) of the influenza hemagglutinin protein to...

...Lysates were prepared from HepG2 cells transfected with NIP45-HA, NF-ATp, or both plasmids, as indicated. Samples indicated with (-) indicate cotransfection with corresponding expression *vector* (for NF-ATp) or an out of frame fusion with the epitope tag (NIP45-HA). Top and middle, lysates were immunoprecipitated with antibody to NF...or expression plasmids for NIP45 and NF-AT family members (NF-ATp, NF-ATc, NF-AT3, and NF-AT4) (B9) as indicated (B20) . One representative *assay* for each combination is shown adjacent to a bar graph representing relative CAT activity for each group. (B) NIP45 synergized with NF-ATp and c...

...IL-4 promoter. HepG2 cells were transfected with an IL-4-CAT reporter construct (extending to -732 bp of the IL-4 promoter) and expression *vectors* or controls for NIP45, NF-ATp, and c-maf as indicated. The control for NIP45 was a frame shift mutant at amino acid 13. Controls for NF-ATp and c-maf were the empty expression *vectors* pREP4 and pMEX, respectively (B3) . Representative CAT *assays* and bar graphs are depicted as in (A...

...endogenous IL-4 production. M12 B lymphoma cells were transiently cotransfected with expression plasmids for NF-ATp and c-maf together with NIP45 or pCI *vector* control, and the *concentration* of IL-4 in supernatants harvested 72 hours later was *measured* by enzyme-linked immunosorbent *assay* (ELISA) (B21...

References and Notes:

...was prepared by cloning a 900-bp fragment of murine NF-ATp (B9) spanning amino acids 228 to 520 into the Bam HI site of *vector* pEG202 (B22) . In-frame fusion of the polypeptide sequence was confirmed by DNA sequence analysis. This bait was used to screen a D10 T cell...

...20. Fold induction was calculated by normalizing the CAT activity of cells transfected with the CAT reporter and each parental expression *vector* to one. Values represent the relative amount of CAT expression above this control transfection. All transfections were done at least three times with one representative...

...with 5 (mu) g of each plasmid for 10 min at room temperature prior to to electroporation at 975 (mu) F, 280 V. The expression *vectors* for c-maf (pMEX-Maf/pREP4), NF-AT (pREP4-NF-ATp), and NIP45 are those used in Fig 4. The control for NIP 45 is the empty *vector*, pCI. ELISA was done according to the instructions of Pharmingen except with modification as

described (B3...

12/3,K/17 (Item 15 from file: 370)
DIALOG(R)File 370:Science

00500921 (USE 9 FOR FULLTEXT)
Myc and Max Homologs in Drosophila
Gallant, Peter; Shiio, Yuzuru; Cheng, Pei Feng; Parkhurst, Susan M.; Eisenman, Robert N.
Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle WA 98104, USA.
Science Vol. 274 5292 pp. 1523
Publication Date: 11-29-1996 (*961129*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2937

(THIS IS THE FULLTEXT)
(*961129*)

Text: The transcriptional regulatory proteins encoded by the myc proto-oncogene family have been linked to *multiple* aspects of eukaryotic cell function, including cell cycle progression, differentiation, and apoptosis (B1) . In addition, genetic alterations in the c-, N-, and L-myc family...
...putative Drosophila Myc may have retained the ability to recognize its heterodimeric partner Max, even from evolutionarily distant organisms. We therefore used human Max to *screen* a two-hybrid library prepared from Drosophila cDNAs. This approach yielded seven partial cDNAs, all encoding the same strongly interacting protein, and we subsequently isolated...

...The putative bHLHZip domain of dMyc is likely to mediate its interaction with human Max in the two-hybrid *screen*, as all dMyc clones isolated from the *screen* encoded this domain. Within bHLHZip, the basic region of dMyc is more similar to its counterpart in human c-Myc (69% identity) than to other...

...In a directed yeast interaction *assay*, the bHLHZip region of dMyc showed no detectable association with the bHLH domains of the Drosophila proteins E(spl)m3, Da, Hairy, and Emc, nor...

...dMyc form homodimers (B15) . We therefore hypothesized that Drosophila may encode a Max-related dimerization partner for dMyc. To identify this protein, we performed a *second* yeast two-hybrid *screen* using the bHLHZip of dMyc as bait. These ...To determine whether the bHLHZip domains of dMyc and dMax exhibit E box binding specificity, we used bacterially expressed proteins in electrophoretic mobility shift *assays* (EMSA) with a labeled oligonucleotide probe (CM-1) containing one copy of the CACGTG site (B18) . The dMyc protein alone did not bind CM-1 even at relatively high *concentrations* of protein, whereas dMax alone bound the probe weakly only at high protein *concentrations* (Fig. 2B). By contrast, dMyc and dMax together generated a strong band shift with CM-1 under conditions in which neither protein alone bound. The...

...over labeled CM-1. We then examined whether dMyc-dMax heterodimers could transactivate a CACGTG-containing reporter gene in mammalian cells (B19) . A cytomegalovirus (CMV) *vector* encoding dMyc stimulated the reporter gene in a *concentration*-dependent manner (Fig. 2C). Expression of dMax alone produced no transactivation over background levels; however, cotransfection of dMax with higher *concentrations* of dMyc *vector* significantly enhanced the transactivation potential of dMyc. These findings demonstrate that the ability of Max and Myc to recognize the CACGTG sequence and activate transcription...strong candidate for dmyc. The dm gene has not yet been cloned, but dm.sup(1) is thought to be a hypomorphic mutation

resulting from *insertion* of a gypsy transposable element. This supposition is based on a genetic interaction of dm.sup(1) with su(Hw), as well as hybridization of...

...and in flies heterozygous for the dm.sup(1) mutation. In chromosomes bearing the dm.sup(1) mutation, the dmyc gene contains a gypsy transposon *insertion* in the first intron, 418 nucleotides upstream of the translation initiation site (B15) . The gypsy *insertion* occurs within a six-nucleotide sequence identical to other known gypsy integration sites (B24) . The location of this element within the dmyc coding region strongly ...

...Our results suggest that the altered expression of dmyc in the dm.sup(1) mutant flies is caused by *insertion* of the gypsy element into the dmyc gene. It is formally possible that the gypsy *insertion* influences expression of other genes; however, the location of the *insertion* and the alterations in dmyc expression argue strongly that dmyc is the critical target gene. Interestingly, the degeneration of the egg chamber in dm.sup ...

...transition to columnar epithelium surrounding the oocyte. We speculate that a stage-specific downregulation of dmyc expression in dm.sup(1) due to the gypsy *insertion* results in a loss of the capacity of the follicle cells to grow and migrate. A possibly related effect has been observed in mice, where...Figure Removed
Removed
Figure F2
Caption: Biochemical properties of dMyc and dMax. (A) In vitro association was *assayed* by incubation of bacterially expressed histidine-tagged dMyc and dMax bound to a nickel resin (His-dMyc; His-dMax) with the [.sup(35)S]methionine...

...was determined from cell lysates 48 hours later (B19) . Duplicate samples from two independent experiments are shown. Samples were normalized for expression from a (beta) -*galactosidase* expression *vector*. Results are presented as mean +/- SEM...

References and Notes:
...the yeast strain Y190. As bait we used a human max cDNA fused in frame with the DNA binding domain of GAL4 in the yeast *vector* GBT9 [C. D. Laherty, unpublished data; P. L. Bartel, C. Chien, R. Sternglanz, S. Fields, in Cellular Interactions in Development: A Practial Approach, D. Hartley, Ed. (Oxford Univ. Press, Oxford, UK, 1993), pp. 153-179]. The cDNA library was from Drosophilia third-instar larvae in the *vector* pACT/pSE 1107. From 1.8 x 10.sup(6) primary transformants, seven clones strongly interacted with human Max, all of which were derived from...

...mutant dmyc gene, genomic DNA was prepared from dm.sup(1)/+ flies as in S. M. Parkhurst and V. G. Corces [Cell 41, 429 (1985)]. *Inserts* from clones hybridizing with probes derived from gypsy and from dmyc were analyzed further...17. Histidine-tagged dMyc and dMax proteins were expressed from pET19b *vectors* (Novagen) containing a dMyc cDNA fragment (amino acids 534 to 717) or a dMax cDNA fragment (amino acids 26 to 161), and purified. In vitro transcription and translation were performed as in (B4) using pRc/CMV *vectors* (Invitrogen, San Diego, CA). For in vitro interaction assays, 4 (mu) l of each translated protein was incubated with 2 (mu) l of His-dMyc...
...5 x 10.sup(5) per 6-cm dish) were transfected with 1 (mu) g of CMV-(beta) gal, 2 (mu) g of pGL2M4 [the *vector* pGL2 (Promega, Madison, WI) containing a fourfold reiteration of the sequence CACGTG] and the indicated amount of pRc/CMV-dMyc, pRc/CMV-dMax, and pRc/CMV *vector* to a total of 9 (mu) g. After 48 hours, luciferase activity was determined [F. M. Ausubel et al., Eds., Current Protocols in Molecular Biology...

00500826 (USE 9 FOR FULLTEXT)

PIN: An Associated Protein Inhibitor of Neuronal Nitric Oxide Synthase

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Publication Date: 11-01-1996 (*961101*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2295

(THIS IS THE FULLTEXT)
(*961101*)

...Text: 377 of nNOS and the Gal4 DNA-binding domain (BD) were transformed with a rat hippocampal cDNA library fused to the Gal4 activation domain (AD). *Screening* of ~3 x 10.sup(6) clones resulted in the isolation of a cDNA that encodes a protein, designated PIN (protein inhibitor of nNOS), that...
...0.9-kb transcript present at highest levels in the testes, intermediate levels in the brain, and lowest levels in most peripheral tissues (B10) . We *screened* a rat brain cDNA library (B11) with the PIN cDNA and obtained a 615-base pair (bp) cDNA that contained a 270-bp open reading...

...was located in a context that conformed to the Kozak consensus sequence (B12) . These data revealed that the clone obtained in the yeast two-hybrid *screen* coded for the complete PIN sequence and 25 amino acids from the 5 (prime) untranslated region...

...ADP) ribose (B20) . GST-PIN bound to the resin in the presence of nNOS, whereas GST did not bind (Fig. 2B). In a blot overlay *assay*, radiolabeled GST-PIN selectively bound nNOS from lysates of nNOS-transfected, but not mock-transfected, HEK 293 cells (B20) (Fig. 2C). Finally, physiologic complexes of...cGMP formation in HEK 293 cells cotransfected with PIN and nNOS (B22) . These experiments revealed that PIN suppressed calcium ionophore-stimulated cGMP formation in a *concentration*-dependent manner (Fig. 3A). Transfection of PIN alone did not alter basal cGMP levels or cGMP levels induced by calcium ionophore (B10...

...nNOS form a dimer that remains intact during low-temperature SDS-PAGE in the presence of tetrahydrobiopterin (BH.inf(4)) and arginine (B25) . In this *assay*, nNOS migrated as a monomer after incubation with *concentrations* of GST-PIN that inhibit >90% of nNOS activity (Fig. 4A). Incubation with GST resulted in minimal loss of dimerized nNOS. We next determined the...

...to PIN in vitro (B10) , implying that PIN may prevent dimerization through a mechanism distinct from that of 7-nitroindazole. In the SDS-PAGE stability *assay*, nNOS migrates as a monomer in preparations that include 7-nitroindazole or that lack BH.inf(4) and arginine, but it still migrates as a dimer in gel filtration *assays* (B25) . A *second*, SDS-sensitive dimerization domain within nNOS may permit dimerization in the presence of 7-nitroindazole. Using the yeast two-hybrid system, we have recently found ...

...While this report was under review, a *screen* for genes required for Drosophila oogenesis led to the cloning of Drosophila and human PIN homologs (B27) . In Drosophila, homozygous loss-of-function mutations are embryonic lethal (B27) . These data, along with the extraordinary evolutionary conservation of PIN and our preliminary evidence that it interacts with *multiple* proteins, suggest that it may be a major regulatory protein influencing numerous physiological processes...
...with the indicated Gal4 AD and Gal4 BD plasmids and grown on plates

containing histidine. A typical filter lift (B5) is shown in which (beta) -*galactosidase* activity was detected by the appearance of a dark blue precipitate. pAD-PIN activated lacZ transcription in the presence of the pBD-NOS(2-377) but not control proteins derived from RAFT. (beta) -*Galactosidase* activity correlated with growth on histidine-deficient plates (B10) . (B) Mapping of the PIN-binding domain of nNOS. The Gal4 BD was fused to regions of NOS, and the ability of these proteins to interact with PIN was *assayed* with the yeast two-hybrid *assay*. The relative (beta) -*galactosidase* activity is indicated in the column on the right. (C) Deduced amino acid sequence of PIN. Abbreviations for the amino acid residues ...Samples of the bacterial lysates are included to show the mobilities of the different recombinant proteins. (C) GST-PIN recognizes nNOS in a blot overlay *assay*. Two cyclic AMP-dependent protein kinase A (PKA) sites were *inserted* between the GST moiety and PIN to create a GST-PIN fusion protein that was labeled in vitro with [(gamma) -.sup(32)P]-adenosine triphosphate...

...NO generation by PIN. (A) PIN reduces NO-dependent cGMP elevation in transfected HEK 293 cells. HEK 293 cells were transfected with a nNOS expression *vector* and the indicated *concentrations* of pmyc-PIN (B22) . The pRK5 parent *vector* was also transfected to adjust the total DNA quantity transfected to 13.75 (mu) g per experiment. In each experiment, nNOS amounts were detected by immunoblot and were unchanged (B10) . Cells were treated with 10 (mu) M calcium ionophore A23187 for 1 hour, and cGMP levels were *measured* by radioimmunoassay (Amersham). (B) PIN inhibits the conversion of arginine to citrulline by nNOS in a *concentration*-dependent manner. GST-PIN (circles) and thrombin-cleaved PIN (triangles) were incubated with transfected cell lysates containing nNOS for 1 hour at 37.Deg.C. NOS *assays* were initiated by the addition of CaCl.inf(2), NADPH, and [.sup(3)H]arginine. The percentage of nNOS activity remaining is indicated relative to activity *measurements* with control proteins at the indicated final *concentrations* (B29) . Control activity levels were determined with either GST or thrombin-cleaved BIRK (B23...

...of 160 kD if the sample is boiled before electrophoresis (B25) . nNOS preparations from transfected HEK 293 cells similar to those in Fig. 3B were *assayed* for dimerization by SDS-PAGE. A boiled sample is included to show the expected mobility of the nNOS monomer. Molecular sizes are indicated in kilodaltons...

References and Notes:

...6. Two-hybrid screens and parent *vectors* pPC97 and pPC86 were as described (B5) . Plasmid pBD-NOS(2-377) was constructed by *insertion* of a nNOS polymerase chain reaction (PCR) product encoding amino acids 2 to 377 into the Sal I-Bgl II sites of pPC97, resulting in...cDNA for PIN was obtained by screening a rat brain (lambda) ZAPII cDNA library (Stratagene) with a probe derived from the Sal I-Not I *insert* in pAD-PIN. Library screening was performed according to the directions of the manufacturer...22. A Sal I-Bgl II fragment comprising the entire translated sequence of the *insert* in pAD-PIN was generated by PCR and subcloned into the Sal I-Bam HI site of the cytomegalovirus (CMV)-driven eukaryotic expression *vector* pCMV-myc to generate a fusion protein consisting of an NH.inf(2)-terminal Myc tag followed by a pentaglycine linker and the PIN *insert*. ;

...for the eNOS plasmid; C. Lowenstein for the iNOS plasmid; A. Lanahan and P. Worley for the rat hippocampal cDNA library and the CMV expression *vector*; D. Sabatini for RAFT constructs; N. Cohen for BIRK constructs; A. Snowman and K. Collins for technical assistance; and D. Bredt, J. Huang, D. Sabatini...

12/3,K/19 (Item 17 from file: 370)

00500766 (USE 9 FOR FULLTEXT)

Visualization of Gene Expression in Living Adult Drosophila

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Publication Date: 10-11-1996 (*961011*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 3235

(THIS IS THE FULLTEXT)
(*961011*)

...Abstract: adult structures, Gal4-UAS (upstream activating site) technology was used to visualize patterns of gene expression directly in living flies. A large number of Gal4 *insertion* lines were generated and their expression patterns were studied. In addition to identifying several characterized developmental genes, the approach revealed previously unsuspected genetic subdivisions of...

...Text: y is responsible for the normal pigmentation of adult cuticle and bristles all over the body. Flies mutant for yellow (y.sup(-)) are of yellow *color* and are clearly distinct from the wild type. The y.sup(-) mutations traditionally have been used as gratuitous markers in genetic mosaic experiments and do...

...with respect to the time of puparium formation, and the emerging adults were inspected for y.sup(+) rescue. Heat shocks given at or after the *second* half of the third larval period are able to produce y.sup(+) rescue. All genes known to be involved in the patterning of adult structures...

...not only during late pupal stages when the adult cuticle is being formed but also much earlier when patterning genes are active. Our finding of *insertions* in several known developmental genes (see below) supports this notion...

...Gal4-containing P element (B8) , to produce lines that express Gal4 in a manner that reflects the expression pattern of the genes where the transposon *inserted*. Each *insertion* was tested indiscriminately in a cross with the UAS-y gene construct. Of 1020 *insertions*, 447 (44%) gave partial or total y.sup(+) rescue in adult flies (see Table 1 for details and different classes of expression). However, the majority of the lines failing to give y.sup(+) rescue correspond to *insertions* in which the GAL4 gene is inactive, as more than 90% of them (56 from a sample of 59) also fail to show any embryonic...

...We are interested in the lines with y.sup(+) rescue that is restricted to specific and well-delimited body regions (Table 1). These lines contain *insertions* in genes that may be involved in developmental processes specific to the structures in which they are expressed. Moreover, these lines can provide information about how adult patterns are genetically subdivided. An additional technical advantage of the *screen* is that it provides a collection of Gal4 driver lines that express Gal4 strongly enough to yield detectable adult expression and therefore are very usefulWe isolated 27 lines that express y.sup(+) in a region-specific manner. Nine of them were later found to be *insertions* in known developmental genes (B13) : Distal-less (Dll), caudal (cad), apterous (ap), optomotor-blind (omb), pannier (pnr), and teashirt (tsh). The y.sup(+) rescue in Dll-GAL4 and cad-GAL4 is illustrated (Fig. 1, A to D). In all the *insertions* in characterized genes, we compared their expression with UAS-y and UAS-lacZ with that revealed for these genes by antibody or in situ hybridization... ...Fig. 1D), y.sup(+) and lacZ expression accurately reflect the overall expression patterns of those genes. Thus, we are confident that in most cases our *screen* reports genuine gene expression patterns. The 18 remaining lines appear to define previously uncharacterized genes because they yield unreported expression patterns. These lines show specific...

...The expression of some of these Gal4 *insertions* in the thorax is of interest because it reveals a genetic subdivision that has not previously been noted. One of these (md237) is an *insertion* at the pnr gene; with UAS-lacZ it produces an embryonic and imaginal expression indistinguishable from the expression pattern of pnr (B14) . md237/UAS-y...

...the terminalia. In the notum, the gene labels a territory extending from the midline to a longitudinal straight line defined by the dorsocentral bristles. Another *insertion*, em462, shows y.sup(+) rescue in a territory adjacent to the pnr domain; it is also demarcated by the position of the dorsocentral bristles (Fig...conclusion, our approach has revealed previously unsuspected genetic subdivisions that may control the disposition of pattern elements in the thorax. It may allow a systematic *screen* for identifying other such subdivisions that may underlie the development of the entire adult pattern ...
... Begin Table : Columns 1 - 2 of 2

Caption:
Number and class distribution of the GAL4 *insertion* lines. Each *insertion* was tested for y+ rescue with the UAS-y construct. Only 44% of the lines give adult rescue, but we estimate that only 5% (3...

...to 30 of them would give a specific pattern; adding these to the 447 that give y+ rescue amounts to 47% (477 /1020) of GAL4 *insertions* responding to local enhancers. This is likely to be an underestimate because it does not take into account the possible cases of specific expression in...

...high proportion of lines give apparent specificity for bristle rescue, and in at least some cases it appears to reflect specific functions; two of these *insertions* turned out to be mutant for the gene javelin, which affects only bristles Reference B26 . However, this apparent specificity has to be regarded with caution...

...clearly y+ and the bristles were y-. No further study of these lines was carried out, but we have observed that the 1J3 line, an *insertion* in the hairy (h) locus Reference B8 , belongs to this class. Because h prevents bristle differentiation by acting as a negative regulator of AS -C...

...in different body regions, making it very difficult to draw a coherent image of the y+ rescue. Finally, the category of region-specific lines includes *insertions* in the Dll, pnr, tsh, ap, cad, and omb genes as well as lines defining additional expression patterns such as em462 and others that yield y+ rescue in wings and halteres, some antennal segments, and some parts of the abdomen Reference B13 .

Total number of *insertions* tested	1020
Number of *insertions* showing y+ rescue	447
Complete rescue	143 (32%)
Bristles y+, cuticle y-	113 (25%)
Cuticle y+, bristles y-	54 (12%)
Heterogeneous rescue	110 (25%)
Body-region-specific rescue	27 (6%)

End Table: Columns 1 - 2 of 2

Figure F1
Caption: Two GAL4 *insertions* in known developmental genes Dll and cad. (A) Adult legs of genotype md23/UAS-y showing y.sup(+) rescue in the tibia (ti), basitarsus (bta...

...femur (f) or trochanter (tr), except for a small region at the trochanter-femur joint (not visible at this magnification). The md23 line is an *insertion* in the Dll locus and is also mutant for Dll. md23/UAS-y

flies also show y.sup(+) rescue along the dorsoventral boundary in the...

...of the same genotype. Expression in the head (h) and thoracic (t) segments coincides with that described for the Dll gene (B4) . We found three *insertions* in the Dll locus (B13) (magnification x 100). (C) Posterior abdominal segments and terminalia of an em459/UAS-y male. em459 is an *insertion* in the cad locus. The VI abdominal segment and genitalia (g) are y.sup(-) but the analia (a) is entirely y.sup(+). The rest of...

...em459/UAS-lacZ embryo after retraction of the germ band showing (arrow) characteristic late embryonic expression of cad in parasegment 15 (B25) . We found two *insertions* in the cad locus (B13) (magnification x 100...

...region, which is mainly out of focus. (B) Normal wg expression as indicated by X-Gal staining of a pharate adult carrying the wg-lacZ *insertion*. The (beta) -*galactosidase* ((beta) -Gal) product accumulates on a longitudinal stripe defined on one side by the dc bristles. Note that the wg domain is included within the...

References and Notes:
...yellow cDNA by cloning two Eco RI fragments [P. K. Geyer, C. Spana, V. G. Corces, EMBO J. 10, 2657 (1986)] into a Bluescript M13+ *vector*. The *vector* was then digested with Not I and Kpn I, and the resulting fragment was subcloned in pUAST (B8) . The DNA was injected into y.sup...

...11. To obtain additional *insertions*, we used the same line carrying the pGawB transposon in the first chromosome described in (B8) . This *insertion* is homozygous lethal and is balanced with a FM7 chromosome. pGawB/FM7 females were crossed to y.sup(-)w.sup(-); DrP((Delta) 2-3)/TM6B...

...5-bromo-4-chloro-3-indolyl (beta) -d-galactopyranoside) staining in embryos was performed as described in (B8) , but sometimes we used antibody to (beta) -*galactosidase* (anti- (beta) -Gal) and standard methods. For better staining of adult cuticle with X-Gal, pharate adults were extracted from the puparium and treated as

12/3,K/20 (Item 18 from file: 370)
DIALOG(R)File 370:Science

00500608 (USE 9 FOR FULLTEXT)
Exclusion of Int-6 from PML Nuclear Bodies by Binding to the HTLV-I Tax Oncoprotein
Desbois, Christelle; Rousset, Raphael; Bantignies, Frederic; Jalinot, Pierre
Laboratoire de Biologie Moleculaire et Cellulaire, Centre National de la Recherche Scientifique UMR49, Ecole Normale Superieure de Lyon, 46 Allee d'Italie, 69364 Lyon Cedex 07, France.
Science Vol. 273 5277 pp. 951
Publication Date: 8-16-1996 (*960816*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 1954

(THIS IS THE FULLTEXT)
(*960816*)

...Abstract: DNA (cDNA) encoding the human Int-6 protein. In mice, the Int-6 gene can be converted into a putative dominant negative oncogene after retroviral *insertion*. Here, Int-6 was localized in the cell nucleus to give a speckled staining pattern superposed to that of the promyelocytic leukemia (PML) protein. The...

...Text: for the mouse mammary tumor virus (MMTV) (B7) . Because it

results in the expression of truncated Int-6 proteins, it is possible that the MMTV *insertion* generates dominant negative oncoproteins. The deduced amino acid sequence of the human protein was identical to that of the mouse form, with no significant homology...

...HeLa cells were transfected with a reporter plasmid containing the gene encoding chloramphenical acetyltransferase (CAT) under the control of GAL4 sites, together with two expression *vectors*: Tax fused to the GAL4 DNA binding domain (GAL4-Tax) and Int-6 fused to the VP16 activation domain (VP16-Int-6). The fusion protein...

...Int-6 were coexpressed (Fig. 1B). Finally, a direct protein-protein interaction was demonstrated by a coimmunoprecipitation assay. COS7 cells were transiently transfected with expression *vectors* encoding Tax and Int-6 fused to the FLAG epitope at its NH.inf(2)-terminus. Immunoprecipitations followed by immunoblotting with antibodies specific for the...

...Tax inside cells. COS7 cells were transfected with a plasmid expressing Int-6 tagged with the FLAG epitope, either alone or with a Tax expression *vector*. Cells were then analyzed by immunofluorescence (B9) . Int-6 was predominantly nuclear and gave a speckled staining pattern (Fig. 3 A). The punctate nuclear localization...next asked whether Tax redistributed specifically Int-6 or had a similar effect on another POD component, like PML itself. COS7 cells were transfected with *vectors* expressing PML and Tax. No overlap in the localization of the two proteins was observed, and Tax did not alter the speckled staining pattern of...

...promyelocytic leukemia (APL) (B13) (B17) . In APL cells, the PML-RARa fusion protein is predominantly localized in the cytoplasm, whereas a fraction remains nuclear within *multiple* smaller clusters (B18) . This abnormal localization is reversed by retinoic acid treatment (B14) (B15) (B18) . A cytoplasmic redistribution of PML has also been observed in...

...Specific association of Tax with Int-6. (A) Analysis of the interaction between Int-6 and Tax or unrelated proteins by a liquid culture (beta) -*galactosidase* assay. SFY526 cells were transformed with Int-6 clone 70 together with plasmids expressing the GAL4 DNA binding domain (GB) alone (pGB-T9) or fused...

...with pVA3 and pTD1, which encodes a protein consisting of GB fused to the SV40 large T antigen (amino acids 84 to 708). The (beta) -*galactosidase* assay with O-nitrophenyl- (beta) -d-galactoside (ONPG) as substrate was carried out on two independent colonies per transformation. The mean values expressed in Miller...

...and Int-6 in a HeLa cell two-hybrid system. Cells were transfected with a CAT reporter construct containing GAL4 sites together with two expression *vectors*: one encodes GB alone (GAL4) or fused to Tax (GAL4-Tax) and the other encodes the VP16 trans-activation domain alone (VP16) or fused to...

...Figure Removed
Removed

Figure F2
Caption: Northern blot analysis of human Int-6 mRNA. (A) Northern blots (Clontech) of *multiple* human tissues containing 2 (mu) g of polyadenylated RNA

References and Notes:
...two-hybrid system (Clontech, Palo Alto, CA). The Tax coding sequence was fused to the GAL4 DNA binding domain into the pGB-T9 yeast expression *vector*, giving pGB-Tax. The human cDNA library we used has been described (B5) . Briefly, cDNAs from EBV-transformed human peripheral lymphocytes were fused to the GAL4 transcriptional activation domain into the (lambda) ACT phage *vector*. pGB-Tax was introduced into the HF7c yeast strain, and the resulting cells were transformed by the fusion cDNA

library. The HF7c strain possesses the...

...of 250,000 transformants, 700 were found to grow on a minimal medium lacking His. Of these, 94 were also positive when assayed for (beta) -*galactosidase* expression. Plasmid DNAs from the double positive clones were extracted and sequenced...fused to the SV40 nuclear localization signal and to the VP16 activation domain (amino acids 403 to 479). The clone 70 Int-6 cDNA was *inserted* into this parental plasmid to give pSG-FNV-70. Duplicate transfections were done for each combination of plasmids. Forty hours after transfection, *concentrations* of the CAT protein were *measured* by enzyme-linked immunosorbent *assay* (Boehringer Mannheim...

...23. Complementary DNA from the Int-6 clone 88 was *inserted* in-frame with the FLAG epitope into pSG5, giving pSGF-Int-6. COS7 cells (0.7 x 10.sup(6)) were transfected with 1 (mu...

...antiserum to Tax; A. Dejean for the PML rabbit polyclonal serum; R. van Driel for the PML monoclonal antibody; P. Chambon for the PML expression *vector*; C. Souchier for help with confocal microscopy; F. Chatelet for assistance in preparing the figures; and J. Maryanski for critical reading of the manuscript. This...

12/3,K/21 (Item 19 from file: 370)
DIALOG(R)File 370:Science

00500500 (USE 9 FOR FULLTEXT)
Evidence for Physical and Functional Association Between EMB-5 and LIN-12 in Caenorhabditis elegans
Hubbard, E. Jane Albert; Dong, Qu; Greenwald, Iva
E. J. A. Hubbard and Q. Dong, Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA. ; I. Greenwald, Department of Biochemistry and Molecular Biophysics and Howard Hughes Medical Institute, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA.
Science Vol. 273 5271 pp. 112
Publication Date: 7-05-1996 (*960705*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2625

(THIS IS THE FULLTEXT)
(*960705*)

...Text: intrinsic signal-transducing activity and when expressed alone are localized in the nucleus, have led to models proposing that there is no intervening cascade of *second* messengers involved in signal transduction initiated by LIN-12/Notch activation. Instead, upon receptor activation, the intracellular domain may influence target gene expression by activating ...observations support this interpretation. First, temperature-shift experiments indicate that emb-5 activity (B21) , like glp-1 activity (B3) , is required continuously for germline development. *Second*, genetic mosaic analysis indicates that emb-5, like glp-1 (B3) , functions in the germ line (Fig. 1B). Finally, even when GLP-1 is ectopically...

...DNA binding hybrid) and (ii) a plasmid
encoding a fusion protein between the Gal4p activation domain (GAD) and another sequence (activation hybrid). A blue colony *color* in the filter *assay* indicates that (beta) -*galactosidase* ((beta) -Gal) activity is present, implying a physical association between the DNA binding and activation domain hybrids as a result of *inserted* sequences. nd, not done.

DNA binding Acti-vation Colony *color* (beta) -Gal

hybrid	hybrid		activity (Miller units)
LIN-12	EMB-5	Blue	4.7 +/- 0.6
GLP-1	EMB-5	Blue	5.3 +/- 0.2...Lamin
EMB-5	White	nd	
FEM-1	EMB-5	White	nd
SNF1	SNF4	Blue	28.5 +/- 4.5

Footnote:
DNA binding hybrids in the pAS1 *vector* Reference B13 contain sequences fused to GAL4p(1-147). LIN-12 = pASL2, containing LIN-12(940-1320). GLP-1 = pASG2, containing GLP-1(867-1171...

...pAS-FEM-1 encodes a hybrid containing the CDC10-SWI6 motifs of FEM-1 Reference B26 .
Footnote:
EMB-5 activation domain hybrids in the pACT *vector* contain sequences fused to GAL4p(768-881) Reference B13 . For EMB-5, the data are from the E7 clone, which contains EMB-5(894-1521...

12/3,K/22 (Item 20 from file: 370)
DIALOG(R)File 370:Science

00500443 (USE 9 FOR FULLTEXT)
Compensatory ahpC Gene Expression in Isoniazid-Resistant Mycobacterium tuberculosis
Sherman, David R.; Mdluli, Khisimuzi; Hickey, Mark J.; Arain, Taraq M.; Morris, Sheldon L.; Barry,III, Clifton E.; Stover, C. Kendall
D. R. Sherman, M. J. Hickey, T. M. Arain, C. K. Stover, Laboratory of Tuberculosis and Molecular Microbiology, PathoGenesis Corporation, 201 Elliott Avenue West, Seattle, WA 98119, USA. ; K. Mdluli and C. E. Barry III, Tuberculosis Research Unit, Laboratory of Intracellular Parasites, National Institutes for Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, MT 59840, USA. ; S. L. Morris, Laboratory of Mycobacteria, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892, USA.
Science Vol. 272 5268 pp. 1641
Publication Date: 6-14-1996 (*960614*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2305

(THIS IS THE FULLTEXT)
(*960614*)

...Abstract: effects of organic peroxides. To survive during infection, isoniazid-resistant KatG mutants have apparently compensated for the loss of KatG catalase-peroxidase activity by a *second* mutation, resulting in hyperexpression of AhpC

...Text: To evaluate the interaction of INH and H.inf(2)O.inf(2) in TB-complex bacilli, we simultaneously administered subinhibitory *concentrations* of both H.inf(2)O.inf(2) and isoniazid to Mycobacterium bovis BCG (an avirulent member of the TB complex). As in Gram-negative...

...saprophyte Mycobacterium smegmatis (B13) , synergy was readily apparent. A reduction in cell viability by a factor of 100 was noted after 72 hours exposure to *concentrations* of H.inf(2)O.inf(2) and INH that separately had no effect in this *assay* (Fig. 1). When subinhibitory *concentrations* of both agents were supplied to a katG-deleted BCG strain, no synergy was observed (B14) . The observed synergy is consistent with the suggestion that...clinical isolates in that they have never experienced passage through animals or liquid culture. The compensatory mutations that up-regulate ahpC expression apparently require a *second* selection event

during infection or further passage in culture...
...in this organism (B20) . To test the role of AhpC in the tubercle bacilli, we cloned ahpC from MTB strain Erdman into a multicopy plasmid *vector* and tested transformants (H37Rv:pMH91) for sensitivity to cumene hydroperoxide. In the context of the supercoiled multicopy plasmid *vector* pMH91, ahpC gene expression was even higher than that seen from the clinical INH-resistant KatG-mutant strains (Fig. 3C). The AhpC-overexpressing strain was...

...smegmatis, BCG, or H37Rv, on plates or in liquid media. Under no condition was the ahpC locus from a clinical isolate able to induce a *measurable* level of INH resistance in the absence of a katG mutation (B14) . Furthermore, we have yet to observe AhpC hyperexpression in passaged INH-resistant strains...

...INH-resistant MTB during infection in vivo to mitigate the added burden imposed by organic peroxides on these strains. Considered by many an old, unsophisticated *antibiotic*, INH may be more properly considered a prototype for new antimicrobials that interact with specific bacterial ... 2)O.inf(2) in 7H9 (no cat) at 37.Deg.C for 1 hour, followed by 4 hours of exposure to the cumene hydroperoxide *concentrations* shown. Control cells (*) were treated identically, except that pretreatment did not include any H.inf(2)O.inf(2). Cell viability was determined by monitoring...
...clinical isolates reveals up-regulation of AhpC. Isoelectric focusing was done in the first (horizontal) dimension, and SDS-polyacrylamide gel electrophoresis was done in the *second* (vertical) dimension. Molecular size standards are shown on the left (in kilodaltons), and isoelectric point (pI) standards are shown along the bottom. (A) Wild-type...

...kb Not I-Pst I fragment containing a 459-bp upstream sequence and the complete AhpC open reading frame (ORF) from MTB strain Erdman was *inserted* into an extrachromosomal shuttle *vector* and transformed into MTB strain H37Rv...

...wild-type M. tuberculosis against cumene hydroperoxide, but not against INH. The fidelity of each construct was confirmed by sequence analysis. (A) MTB H37Rv:pMV206H (*vector* control) (*) and MTB H37Rv:pMH91 ((open-circle)) were grown to mid-log phase and then diluted to an A.inf(650) of 0.05, and...

References and Notes:
...675-bp sequence upstream of the katG gene of MTB strain Erdman was used to drive expression of the lux gene in an integrating shuttle *vector*. Fidelity of constructs was determined by sequence analysis. Transformations into BCG and lux assays were done as described (B23...

12/3,K/23 (Item 21 from file: 370)
DIALOG(R)File 370:Science

00500346 (USE 9 FOR FULLTEXT)
HIV-1 Entry Cofactor: Functional cDNA Cloning of a Seven-Transmembrane, G Protein-Coupled Receptor
Feng, Yu; Broder, Christopher C.; Kennedy, Paul E.; Berger, Edward A.
Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD 20892, USA.
Science Vol. 272 5263 pp. 872
Publication Date: 5-10-1996 (*960510*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 4478

(THIS IS THE FULLTEXT)
(*960510*)

...Text: We previously reported a recombinant vaccinia virus-based transient expression and *assay* system in which fusion between Env-expressing and CD4-expressing cells leads to activation of a reporter gene (Escherichia coli lacZ) (B12) . We adapted this...
...NIH 3T3 cells expressing vaccinia-encoded T7 RNA polymerase and CD4 were transfected with a HeLa cDNA plasmid library (*inserts* linked to the T7 promoter). These cells were mixed with NIH 3T3 cells expressing vaccinia-encoded Env and containing the Escherichia coli lacZ gene linked to the T7 promoter. After incubation, the cultures were stained for (beta) -*galactosidase* ((beta) -Gal) in situ. Consistently more (beta) -Gal-positive cells were observed with the CD4-expressing cells transfected with the entire library compared to control...

...of the gp120/gp41 cleavage cite. These results suggested that the library contained at least one cDNA encoding a fusion cofactor. After repeated subfractionation and *screening*, we isolated individual colonies on agar plates, and a single plasmid clone was identified that was capable of allowing the CD4-expressing NIH 3T3 cells to undergo fusion. The size of the cDNA *insert* was ~1.7 kb...

...DNA sequence analysis was performed on both strands of the *insert*. The cDNA contained 1659 base pairs, and the longest open reading frame of the coding strand was 352 amino acids (B16) . Analysis of this sequence...

...The cDNA *insert* was cloned (B23) into plasmid pSC59 (B24) , which contains a strong synthetic vaccinia promoter supporting early and late transcription, flanked by sequences of the gene...As a *second* approach for testing the functional activity of recombinant fusin, we selected fusin transformants of a CD4-expressing nonhuman cell type and tested them for susceptibility...

...We transfected the CD4.sup(+) Mv 1 Lu transformant with a plasmid *vector* containing the fusin cDNA linked to the SV40 promoter (as well as a gene conferring resistance to Zeocin); we transfected control cells with the same plasmid *vector* containing an irrelevant gene (lacZ) linked to the SV40 promoter. After selection in the presence of both G418 and Zeocin, individual colonies were tested for fusion with cells expressing Env by the vaccinia expression-*assay* system (using luciferase as the reporter); all colonies were positive (B29) . The three colonies with the best fusion activity were examined for susceptibility to HIV...

...that p24 production represented true productive infection (B29) . The fusin-CD4 mink cell transformants were sufficiently susceptible to HIV-1 infection to enable direct p24 *measurement* without the need for cocultivation with permissive human target cells. However, the infection efficiency for the mink cell transformants was considerably less than that for...
...cell types does not allow HIV-1 infection, whereas the corresponding CD4.sup(+) transformants do (B1) (B3) . Thus, as shown above in the cell fusion *assay*, fusin promotes HIV-1 infection by serving as a cofactor in CD4.sup(+) cells...cell line), a prominent RNA band at ~1.7 kb was detected; the size of this transcript is consistent with the length of the cDNA *insert* and is similar to that previously reported for various human cell types (B18) (B19) (B20) (B21) . By contrast, this transcript was not detected in the...

...type-specific fusion cofactors (B35) . We predict that fusin should function preferentially for Envs from T cell line-tropic isolates, because it was identified by *screening* a cDNA library from a human continuous cell line (HeLa) for the ability to allow a T cell line-tropic Env (Lai, IIIB) to undergo...

...acts directly as a coreceptor in the fusion process. This could occur by interaction either with Env (perhaps via the V3 loop) or with CD4; *multiple* and possibly sequential interactions can be readily imagined. Alternatively, the role of fusin in entry might be indirect, possibly involving G protein signaling...undergo Env-CD4-mediated cell fusion. Cell

fusion mediated by vaccinia-encoded proteins was analyzed by syncytia formation and by the quantitative reporter gene activation *assay* (B12) . NIH 3T3 cells were co-infected with vTF7-3 (encoding T7 RNA polymerase) (B49) plus the designated combinations of vCB-3 encoding CD4 (B6...

...hours at 37.Deg.C, samples on one plate were fixed, stained with crystal violet, examined microscopically for syncytia, and photographed. Samples (duplicates) on the *second* plate were treated with Nonidet P-40 (0.5% final *concentration*), and 50- (mu) l aliquots of each sample were mixed with 50 (mu) l of 2 x substrate solution (chlorphenol red- (beta) -d-galactopyranoside) in 96-well plates. We monitored the rates of substrate hydrolysis at ambient temperature by *measuring* absorbance at 570 nm using a microplate absorbance reader (Molecular Dynamics). Values (insets) represent absorbance/min multiplied by 10.sup(3) (mean of duplicate samples...

References and Notes:
...15. A HeLa cell cDNA library cloned unidirectionally into the plasmid *vector* pcDNA3 with the cDNAs linked to the T7 promoter was purchased from Invitrogen; the library was derived from 4 x 10.sup(6) clones. For ...23. Plasmid pcDNA3-fusin was digested with Xho I and blunt-ended with the Klenow fragment of E. coli DNA polymerase. The fusin cDNA *insert* was excised by digestion with Eco RI and ligated into Stu I-Eco RI-digested pSC59 (B24) . The resulting plasmid, designated pYF1-fusin, was used...28. The fusin cDNA *insert* was excised from pCDNA3-fusin with Hind III and Xho I and ligated to Hind III-Xho I-digested pZeoSV (Invitrogen). The resulting plasmid (pZeoSV...

...Zeocin resistance gene. After 24 hours, the cells were washed and cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum plus *antibiotics* (during the first 2 days with 1 mg/ml of G418 alone; subsequently, with G418 plus 1 mg/ml of zeocin). Colonies resistant to both *antibiotics* were picked, amplified, and screened for Env-dependent fusion permissiveness in the vaccinia assay system (with the use of luciferase as the reporter...

12/3,K/24 (Item 22 from file: 370)

00500268 (USE 9 FOR FULLTEXT)
In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral *Vector*
Naldini, Luigi; Blomer, Ulrike; Gallay, Philippe; Ory, Daniel; Mulligan, Richard; Gage, Fred H.; Verma, Inder M.; Trono, Didier
L. Naldini, U. Blomer, P. Gallay, F. H. Gage, I. M. Verma, D. Trono, Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. ; D. Ory and R. Mulligan, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA.
Science Vol. 272 5259 pp. 263
Publication Date: 4-12-1996 (*960412*) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 3384

(THIS IS THE FULLTEXT)
In Vivo Gene Delivery and Stable Transduction of Nondividing Cells by a Lentiviral *Vector*
(*960412*)
Abstract: A retroviral *vector* system based on the human immunodeficiency virus (HIV) was developed that, in contrast to a murine leukemia virus-based counterpart, transduced heterologous sequences into HeLa cells and rat fibroblasts blocked in the cell cycle, as well as into human primary macrophages. Additionally, the HIV *vector* could mediate stable in vivo gene transfer into terminally differentiated neurons. The

ability of HIV-based viral *vectors* to deliver genes in vivo into nondividing cells could increase the applicability of retroviral *vectors* in human gene therapy

Text: Until now, gene therapy protocols have often relied on *vectors* derived from retroviruses such as murine leukemia virus (MLV) (B1) (B2) . These *vectors* are useful because the genes they transduce are integrated into the genome of the target cells, a desirable feature for long-term expression. However, these retroviral *vectors* can only transduce dividing cells, which limits their use for in vivo gene transfer in nonproliferating cells such as hepatocytes, myofibers, hematopoietic stem cells, and neurons (B3) (B4) . The optimal gene transfer system would include a retroviral *vector* based on a virus, such as HIV and other lentiviruses, that can integrate into the genome of nonproliferating cells. In vitro, HIV can infect primary...
...A three-plasmid expression system was used to generate HIV-derived retroviral *vector* particles by transient transfection, as described for other *vectors* (B10) (Fig. 1). Plasmid pCMV (Delta) R9, the packaging construct, contains the human cytomegalovirus (hCMV) immediate early promoter, which drives the expression of all viral...

...eliminated cis-acting sequences crucial for packaging, reverse transcription, and integration of transcripts derived from the packaging plasmid (B12) . To broaden the tropism of the *vector*, we used a *second* plasmid that encodes a heterologous envelope protein for pseudotyping the particles generated by pCMV (Delta) R9 (B13) . Two variants of this construct were used: One...

...encodes the G glycoprotein of vesicular stomatitis virus (VSV G) (B14) . The latter envelope offers the additional advantage of high stability, which allows for particle *concentration* by ultracentrifugation (B15) . The third plasmid, the transducing *vector* (pHR (prime)), contains cis-acting sequences of HIV required for packaging, reverse transcription, and integration, as well as unique restriction sites for the cloning of...

...base pairs of gag as well as env sequences encompassing the Rev response element (RRE) flanked by splice signals were included in the pHR (prime) *vector* (B16) . This design had a dual purpose: first, to increase packaging efficiency, as both gag and env RNA determinants have been demonstrated to enhance this process (B17) , and *second*, to allow the efficient transcription and cytoplasmic export of full-length *vector* transcripts only in the presence of the HIV Tat and Rev regulatory proteins, both of which are encoded by the packaging plasmid, pCMV (Delta) R9. In the absence of these transacting factors, the only detectable expression originated from the internal promoter in the *vector* (B18) . The Escherichia coli (beta) -*galactosidase* ((beta) -gal) or the firefly luciferase coding sequences were *inserted* into pHR (prime) downstream of the hCMV immediate early promoter to serve as reporter genes...

...Replication-defective retroviral particles were generated by transient cotransfection of 293T human kidney cells with the three-plasmid combination (B19) . MLV-derived packaging and transducing *vectors* served as controls (B20) . Media from the various transfectants were first *assayed* for transduction frequency on growing 208F rat fibroblasts (B21) . HIV-based (beta) -gal *vectors* yielded titers of 0.8 (+/-1.7) x 10.sup(5) (n = 3) transducing units (TU) per milliliter with the MLV(Ampho) envelope and 4 (+/-1.5) x 10.sup(5) (n = 6) TU/ml with the VSV envelope. These titers are comparable with those obtained with MLV-based *vectors* produced by the same method-10.sup(5) TU/ml with its own envelope, and 5 x 10.sup(5) Tu/ml when pseudotyped with the VSV envelope-and significantly higher than those previously reported for other HIV-based *vectors* (B17) (B22) . Potentially contributing to this increased efficiency is the incorporation of accessory HIV-1 genes into the packaging construct, including nef that markedly enhances...

...The HIV-derived *vector* system used here is devoid of helper virus per se. Furthermore, the use of a three-plasmid combination and of a

heterologous envelope, as well as the removal of *multiple* cis-acting sequences from the packaging *vector*, makes it unlikely that a replication-competent recombinant would be generated. The potential transfer of packaging functions from producer to target cells was *assayed* by testing for the production of the tat and gag gene products in *vector* -transduced cells. Neither protein was detected, which, considering the sensitivity of the *assays* we used (B24) , implied that the transfer of packaging functions was at least three orders of magnitude less efficient than that of *vector* sequences. Furthermore, conditioned medium from serially passaged transduced cells did not transfer the reporter gene to naieve cells (B24...

...HIV-and MLV-derived *vectors* were compared for their ability to transduce cells blocked at various stages of the cell cycle. HeLa cells were growth-arrested at the G.inf...

...the cells at the time of infection was verified by propidium iodide staining of the DNA and by flow cytometry (B18) . An HIV-based retroviral *vector* expressing (beta) -gal was as efficient at transducing G.inf(1)-S- and G.inf(2)-arrested as proliferating HeLa cells, whereas its MLV counterpart...

...To test whether the HIV-based *vector* integrates in the host cell genome, we used packaging constructs carrying mutations that inactivate integrase. HIV-1 mutants in which the expression of integrase is...

...end do not reverse transcribe their genome efficiently (B26) . When this mutation was introduced into the packaging construct, it completely prevented transduction by the resulting *vector* particles. Furthermore, whereas a (beta) -gal *vector* made with the wild-type packaging construct had a transduction efficiency of 940 TU per nanogram of p24 in growing or G.inf(1)-S...

...reverse transcription as well as integration. Taken together, these results indicate that the unique features of HIV can be transferred to a replication-defective retroviral *vector*, allowing transduction of nonproliferating cells...

...to confluence and then maintained them in G.inf(0) by density-dependent inhibition of growth in the presence of dexamethasone (B3) . The HIV-based *vector* was significantly more efficient than its MLV equivalent. However, its transduction rate decreased as a function of time between growth arrest and infection (Table 1...

...dividing cells. However, in cells that had been maintained in G.inf(0) for 15 days, the relative transduction decreased to 17%. The MLV-based *vector* was significantly more affected by the growth arrest. In its case, the residual transducing activity reflected the fraction of cells still undergoing division, as assessed by propidium iodide staining of the cell DNA followed by flow cytometry (B29) . Whereas *vector* particles entered G.inf(0)-arrested and dividing cells with comparable efficiencies (B30) , they were significantly defective for reverse transcription in G.inf(0) cells...

...50% of those obtained in dividing cells (Table 1). In contrast, inducing cell division even 1 day after inoculation did not rescue the MLV-derived *vector*. The generation of a stable infection intermediate by the HIV-based *vector* offers an advantage for delivering genes into targets such as hematopoietic stem cells. Indeed, it may alleviate the need for inducing the proliferation of these...

...The decreased transduction efficiency of the HIV *vector* in G.inf(0)-arrested fibroblasts may partly reflect suboptimal *concentrations* of intracellular deoxynucleotides (B32) . Whether a similar limitation would preclude gene transfer into terminally differentiated primary cells could not be inferred from these observations and was therefore assessed directly. The HIV-based luciferase *vector*,

pseudotyped with the VSV G protein, was tested for its ability to transduce human monocyte-derived primary macrophages (B33) . Significant levels of luciferase activity were detected in an envelope-dependent manner (Table 2). In contrast, only background levels of luciferase activity were *measured* in macrophages inoculated with a comparable VSV G-pseudotyped MLV-based *vector* (B34) . To rule out that the HIV *vector* was infecting a small proportion of macrophages that were proliferating, we generated mutant packaging constructs where Vpr and the nuclear localization signal (NLS) present in ...of these two elements is essential for viral infection in macrophages, because they mediate nuclear import of the HIV preintegration complex (B7) (B8) (B9) . A *vector* assembled from a mutant packaging construct in which both Vpr and the MA NLS are inactivated was severely reduced in its ability to transduce macrophages (Table 2). Similarly, NLS peptide treatment prevented transduction by a *vector* produced from a Vpr-defective packaging construct, thus corroborating the previously demonstrated inhibition of MA-mediated nuclear import of the HIV preintegration complex by this peptide (B9) . Neither MA-Vpr double mutations nor NLS peptide treatment affected the ability of the *vectors* to transduce dividing cells (B18) . The requirement for interaction with the cellular nuclear import machinery, together with the lack of significant transduction by the MLV *vector*, demonstrates that gene transfer by the HIV *vector* did occur in nonproliferating macrophages and not simply in a small proportion of dividing cells in the culture...

...To test if HIV-based *vectors* can deliver genes in vivo, we injected highly concentrated stocks of HIV-or MLV-based (beta) -gal *vectors* pseudotyped with VSV G protein bilaterally into the corpus striatum and hippocampus of adult female rat brains (B36) . Seven or 30 days later the brains...

...apparent 1 month after the injection. Areas of (beta) -gal-positive cells were detected surrounding all injected sites for both HIV-based and MLV-based *vectors*. In brains injected with the HIV-based *vector*, a variety of (beta) -gal-positive cells with a morphology resembling neurons, oligodendrocytes, and astrocytes could be detected (B18) . To further identify the cell types transduced by both *vectors*, we used confocal microscopy after immunofluorescence staining with antibodies specific for (beta) -gal, glial fibrillary acidic protein (GFAP, a marker for astrocytes), and NeuN (a marker for terminally differentiated neurons) (B37) . Sections from brains injected with the MLV-based *vector* contained cells either labeled only for (beta) -gal or for both (beta) -gal and GFAP (B18) . The MLV *vector* was unable to transduce neurons because no cells labeled for both (beta) -gal and NeuN were detected. In contrast, the striatum of animals injected with the HIV-based *vector* showed *multiple* cells double-labeled for (beta) -gal and NeuN (Fig. 3 , top panel), demonstrating the ability of the HIV-based *vector* to infect and transduce genes in terminally differentiated neurons. NeuN and (beta) -gal double-positive cells were also detected in the hippocampus of brains injected with the HIV *vector*. As expected, the HIV-based *vector* was also able to transduce astroglial cells (B18) . The expression of (beta) -gal in neurons in the striatum and the hippocampus could be detected after ...

...Our results lend strong credence to the idea that HIV-based *vectors* transduce genes efficiently and can be used for in vivo gene delivery. Because retroviruses integrate in the genome of the target cells, repeated transduction is unnecessary. Therefore, in contrast to an adenoviral *vector* capable of in vivo gene delivery, problems linked to the humoral response to injected viral antigens can be avoided (B38) . Furthermore, the *vectors* described here are replication defective; consequently, the transduced cells lack viral protein that could trigger a cellular immune response. A major goal of our work was to establish a proof of principle that lentiviral *vectors* can be used for stable in vivo gene delivery in nondividing cells. For human experimentation, it may be more prudent to develop *vectors* derived from nonhuman lentiviruses such as simian immunodeficiency virus, bovine immunodeficiency virus, or equine infectious anemia virus. We believe that the generation of safe and efficacious

lentiviral *vectors* will significantly advance the prospects of human gene therapy...

...Figure F1
Caption: Schematic representation of the HIV provirus and the three-plasmid expression system used for generating a pseudotyped HIV-based *vector* by transient transfection. Only the relevant portion of each plasmid is shown. For the HIV provirus, the coding region of viral proteins, including the accessory...

...and a SV40 poly(A) site. The VSV G coding region is flanked by the CMV promoter and a poly(A) site. In the transfer *vector* pHR (prime) , the gag gene is truncated and out of frame (X), and the internal promoter CMV is used to drive expression of either (beta) -*galactosidase* (lacZ) or luciferase cDNA. The Rev responsive element (RRE) and splice acceptor site (SA) are shown...Removed

Begin Table : Columns 1 - 3 of 3

Caption:
Relative transduction of cells at different stages of the cycle by HIV- and MLV-based *vectors*. Results are expressed relative to the transduction obtained by the *vector* in growing cells. Multiplicity of infection was matched for both *vectors*. Abbreviations: arr., arrested; d, days; repl., replated.

Infected culture	Tranduction efficiency	
	HIV-based *vector*	MLV-based *vector*
HeLa cells		
Growing	1	1
G1-S-arr.	0.97 +/- 0.02	0.05 +/- 0.01
G2-arr.	0.71 +/- 0.22	0.08 +/- 0...

...by aphidicolin treatment or in G2
by exposure to 40 grays (1 gray = 100 rads) of gamma radiation Reference B25 and infected with (beta) -gal *vector* pseudotyped with MLV (Ampho) envelope. Transduction was scored by X-Gal staining of the cultures 48 hours after infection. Results are the mean +/- SEM determination...

...medium
containing 5% calf serum and 2 (mu) M dexamethasone Reference B3 , and further incubated for the indicated number of days before infection with luciferase *vectors* pseudotyped with VSV G protein. Transduction was scored by measuring luminescence in cell extracts 48 hours after infection. Results are the mean +/- SD of replicated...

...experiment of a total of five performed.
Footnote:
Rat 208F fibroblasts either growing or arrested in G0 for 3 weeks were infected with (beta) -gal *vectors* pseudotyped with the MLV (Ampho) envelope. Transduction was scored by X-Gal staining either 48 hours after infection (growing and G0) or 48 hours after...

...a total of four performed.
End Table: Columns 1 - 3 of 3

Figure F2
Caption: Reverse transcription and nuclear import of the HIV-based *vector* genome in fibroblasts growing or arrested in G.inf(0). Cultures of 208F fibroblasts were plated at low density and either infected the following day (growing) or grown to confluence and further incubated for the indicated number of days (G.inf(0) X days) before infection with HIV-based luciferase *vector* pseudotyped or not ((Delta) Env) with VSV envelope. At the indicated time in hours after infection, cells were lysed and assayed by PCR with primers...

...DNA) blot with a .sup(32)P-labeled HIV proviral DNA probe. EL, early products (strong stop DNA); LL, late linear products (generated after the *second* template switch); Ci, two-LTR circles (formed in the nucleus...

...4 of 4

Caption:
Transduction of human monocyte-derived macrophages. Primary cultures of human macrophages prepared from different donors were incubated with HIV -based luciferase *vectors* pseudotyped with VSV G protein and generated either from wild-type (pCMV (Delta) R9) or mutant packaging plasmids carrying inactivating mutations in the vpr gene...

...infection,
as previously described Reference B8 . Luminescence was measured in cell extracts 48 hours after infection. Transduction was dependent on active nuclear import of the *vector* in target cells, as it was inhibited by mutations inactivating Vpr and the MA NLS in the packaging plasmids and when infected cells were incubated
...

...
Figure F3
Caption: The in vivo transduction of adult rat neurons. Confocal microscope images of sections from brains injected with HIV-based (beta) -gal *vectors* stained by immunofluorescence for (beta) -gal, NeuN, and glial fibrillary acidic protein (GFAP). The images obtained from each individual staining and from their overlap are...surrounding the injection site are shown for a section from striatum 1 week and 4 weeks and from hippocampus 4 weeks after injection of the *vector*. Several cells doubly labeled for (beta) -gal and NeuN (arrows) are evident in the sections. The overall pattern was reproduced in all five animals (three examined after 7 days, and two after 30 days) injected with the HIV-based *vector*.

References and Notes:
...pR9, an infectious molecular clone of proviral HIV-1 DNA made by cloning the Bss HII-Bam HI fragment of NL4.3 in pR7, with *insertion* of a Mlu I linker at the Stu I site that frameshifts the env reading frame [D. Trono, M. B. Feinberg, D. Baltimore, Cell 59...low-speed centrifugation, filtered through 0.45- (mu) m filters, and assayed for p24 Gag antigen by enzyme-linked immunosorbent assay (ELISA) (DuPont). The average *vector* yield was 50 to 80 ng of p24 per milliliter...

...20. MLV-based *vectors* were produced from transient transfection in 293T cells of the following plasmids. pSLX-CMVLacZ [R. Scharfmann, J. H. Axelrod, I. M. Verma, Proc. Natl. Acad. Sci. U.S.A. 88, 4626 (1991)] is a MLV-derived *vector* carrying a hCMV-driven E. coli lacZ gene. The pCL plasmid series carries a hybrid CMV-LTR promoter that allows for CMV-driven transcription in...

...reconstitution of a functional LTR in the target cell (R. Naviaux, E. Costanzi, M. Haas, I. Verma, in preparation). The luciferase gene was cloned in *vector* pCLNCX, creating pCLNCLuc. MLV-based *vectors* with the cognate MLV (Ampho) envelope were produced by the cotransfection of either of the *vector* plasmids with the amphotropic packaging plasmid pCL-Ampho. VSV G-pseudotyped *vectors* were produced by the cotransfection of either of the *vector* plasmids with the MLV gag-pol packaging plasmid pCMV-GAGPOL and the VSV G plasmid...

...foci of blue cells per well and dividing that number by the dilution factor. Transduction of the reporter gene was only observed when the packaging *vector* and Env-coding plasmid had been cotransfected in 293T cells; no transduction was observed when either plasmid was omitted or when the HIV-based *vector* was cotransfected with an MLV-based packaging plasmid or vice versa. Virtually all cells in a well could be transduced

when a multiplicity of infection (MOI) >1 was used. When the luciferase *vector* was used, transduction was assayed by washing the cultures twice with tris-buffered saline (TBS), extracting the cells with 200 (mu) l per well of...

...40 in TBS containing 5 mM MgCl.inf(2), and assaying a 50- (mu) l sample for luminescence in a luminometer. The HIV-based luciferase *vector* transduced 930 +/- 240 (n = 4) relative luminescence units (RLU) per microliter of infected transfectant-conditioned medium with VSV envelope, and 460 +/- 110 (n = 2) RLU with MLV (Ampho) envelope. MLV-based luciferase *vector* pseudotyped with VSV envelope transduced 920 RLU/ (mu) l...24. Presence of helper virus and transfer of the HIV tatgene were measured by inoculating HeLa P4.2 cellswith HIV *vector*, pseudotyped or not with MLV (Ampho) envelope, and staining with X-Gal after 48hours. P4.2 cells express CD4 and contain an integrated lacZ reporter...

...barely detectable activity of 0.023 TU per nanogram of p24, and no transduction of Tat was detected with a maximal dose tested of HIV *vector* corresponding to 1.2 (mu) g of p24 Gag antigen, either with or without envelope. On the other hand, when assayed for the transduction of (beta) -gal into naieve HeLa cells, HIV *vector* pseudotyped with MLV (Ampho) envelope had an average efficiency of 115 TU per nanogram of p24 and of 940 TU/ng when pseudotyped with VSV...

...in extracts of HeLa cultures serially passaged after infection with the viral equivalent of 1 (mu) g of p24 of both MLV (Ampho)-pseudotyped HIV *vector* and virus. The detection limit was >=1 pg per milliliter of extract. Cells infected with envelope-defective, pseudotyped HIV virus consistently gave readings above 20 ng/ml. No Gag antigen was detected in extracts of *vector*-transduced cells. The same held true when HeLa cells transduced with the pHR (prime) -LacZ *vector* were selected for (beta) -gal expression by live fluorescence-activated cell sorting after fluorescein-di- (beta) -d-galactopyranoside (FDG) staining [G. P. Nolan, S. Fiering...Sal I cassette containing the mutations from plasmids p (Delta) INR8 (B9) and pHIV-Hygro-D64V (B27) . Although residual transducing activity was scored for the *vector* assembled from the packaging plasmid carrying the D64V integrase mutation, (beta) -gal-positive cells showed on average significantly weaker staining than those transduced by the wild-type *vector* and were apparently unable to form foci of stably transduced cells. This is also consistent with the residual activity observed for the D64V integrase mutation...

...30. Entry of the HIV-based *vector* in cultures of 208F cells growing and growth-arrested for 21 days was assayed by measuring the envelope-dependent uptake of p24 Gag protein. Cultures were incubated overnight with 33 ng of p24 Gag antigen of HIV-based *vector* either pseudotyped or not, washed, trypsinized, and extracted for measuring p24 content by ELISA. Growing cells contained 553 +/- 50 pg of p24 after incubation with pseudotyped *vector*, and 100 +/- 25 pg of p24 after incubation with particles with no envelope; G.inf(0) cells contained 592 +/- 120 pg of p24 after incubation with pseudotyped *vector*, and 90 +/- 15 pg of p24 after incubation with particles with no envelope...in RPMI containing 10% human serum for 2 to 4 weeks before infection. Cultures were infected without or with HIV-based and MLV-based luciferase *vectors* pseudotyped or not with VSV envelope. For the HIV-based *vector*, 150 ng of p24 equivalent were used per each inoculum...

...T) replace two lysines (K) in the NLS of MA. The construction and biological properties of these mutants have been described (B8) (B9) . Occasionally, mutant *vectors* showed a less pronounced phenotype in the macrophages from one donor, perhaps because of variation in the state of growth arrest of the cells. High...

...36. The details of *vector* preparation will be published elsewhere (L. Naldini et al., in preparation). After concentration by ultracentrifugation, titers of 1 x 10.sup(8) to 3 x...

...75 mg/kg), andxylazine (4 mg/kg) in 0.9% NaCl, intraperitoneally],positioned in a stereotactic head frame, and slowlyinjected with 2 (mu) l of *vector* stock into the striatum[anteroposterior (AP), +0.2; mediolateral (ML),+/-3.5; dorsoventral (DV), -4.5] and hippocampus (AP, -3.5, ML, 3.0; DV...

...hours, saturated in 30% sucrose, and sectioned on a freezingmicrotome (40- (mu) m sections). Light microscopy sections were stained with avidin-biotin peroxidase (Vectastain Elit, *Vector* Labs) and diaminobenzidine. Immunofluorescence triple labeling was conducted with rabbit antibody to (beta) -gal (anti- (beta) -gal) (1:1000, Cortex), mouse monoclonal anti-NeuN (1...39. For the polymerase chain reaction (PCR) assay, cultures were incubated with *vector* concentrated by ultracentrifugation and pretreated with deoxyribonuclease I (DNase I) (20 (mu) g/ml for 2 hours at 37.Deg.C), washed, trypsinized, and extracted...

...to 542 and 9606 to 9626). LTR5 plus LTR6 amplifies minus-strand strong stop DNA, LTR5 plus 5NC2 amplifies double-stranded molecules generated after the *second* template switch, and LTR8 plus LTR9 amplifies two LTR circles. A series of logarithmic dilutions of pHR (prime) plasmid used as a template showed linearity...

12/3,K/25 (Item 1 from file: 444)

00118483

High-Level Chloramphenicol Resistance in Neisseria meningitidis (Original Articles)

Galimand, Marc; Gerbaud, Guy; Guibourdenche, Martine; Riou, Jean-Yves; Courvalin, Patrice.
The New England Journal of Medicine
Sep 24, 1998; 339 (13),pp 868-874
LINE COUNT: 00414 WORD COUNT: 05722

TEXT

...Among the bacteria that cause serious infections, N. meningitidis is one of the least problematic in terms of *antibiotic* resistance. (Ref. 2) Resistance to penicillin G, sulfonamides, rifampin, and tetracyclines has been reported, but high-level resistance to penicillins through (beta)-lactamase production is...supplements, as described by Kellogg et al., (Ref. 11) and were incubated at 37 degreesC with 5 percent carbon dioxide. The minimal inhibitory concentrations of *antibiotics* were determined by the E test on supplement G-containing agar medium (Sanofi Diagnostics Pasteur). Chloramphenicol acetyltransferase and aminoglycoside-modifying enzymes were assayed in supernatants (centrifuged at 100,000 x g) after ultrasonic disintegration. (Ref. 12,13) With Escherichia coli JM83, the following *antibiotics* were used: chloramphenicol (10 mg per liter) for cloning the resistance gene and kanamycin (20 mg per liter) for cloning the PCR product. (Ref. 14) With neisseriae, the following *antibiotics* were used: chloramphenicol (10 mg per liter), nalidixic acid (20 mg per liter), and tetracycline (2 mg per liter). The transformation of N. meningitidis BM4376...in lanes 15 and 16, respectively. DNA was digested by BglII and fractionated by pulsed-field gel electrophoresis (under the following conditions: initial pulse, 1 *second*; final pulse, 10 seconds; voltage, 6 V per centimeter; duration of electrophoresis, 30 hours; included angle, 120 degrees; and temperature, 17 degreesC) (Panel A), transferred...

...from N. meningitidis strain LNP13947 and pUC18 DNA digested with Sau3A and BamHI, respectively, were mixed, ligated, and introduced into E. coli JM83 (minimal inhibitory *concentration* of chloramphenicol, 2 mg per

liter). The resulting transformed isolates (transformants) were selected on the basis of their resistance to chloramphenicol, and the smallest hybrid plasmid, pAT447, was found to contain a 6.5-kb *insert*. Subcloning was accomplished by introducing the 4.6-kb EcoRI-HindIII fragment of the *insert* into pUC18, which generated pAT448. The latter construct conferred high levels of resistance to the new host (minimal inhibitory *concentration*, >256 mg per liter) by synthesis of a chloramphenicol acetyltransferase, as determined by an enzyme *assay* (data not shown...

...truncated transposon and that of the host, two primers were designed on the basis of the 5' and 3' meningococcal sequences flanking the Tn4451-like *insertion* (primer C, 5'CTAAATCAATAATAATATTCCC3', and primer D, 5'ACCCCAGGTAGAATACAATGAA3...

...These primers, which allow amplification of a 1206-bp fragment with the *insertion* of pAT448 as a template (Fig. 2, lane 6), gave rise to products of approximately 1200 bp in the chloramphenicol-resistant isolates (Fig. 2, lanes...respectively. Lanes 1 and 22 show fragments obtained by digestion of bacteriophage lambda DNA by PstI and used as standards for molecular size *.**FIGURE OMITTED**

Insertion Site of the catP Gene into the N. meningitidis Chromosome

The sequence of the catP gene and flanking regions from transposon Tn4451, from the pAT448 *insert*, and from the 1200-bp PCR products obtained from the transformants was compared with that of the 200-bp product obtained from susceptible N. meningitidis strains (Fig. 3). *Insertion* of the catP gene occurred at the same site in a region with a large proportion of adenosine and thymidine (77 percent, as compared with ...

...1-bp or 2-bp deletion in the target DNA or no loss of meningococcal DNA in the transformants (Fig. 3).|*Figure 3.-Site of *Insertion* of the catP Gene into Chromosomal DNA of N. meningitidis.The nucleotide sequence of the flanking regions of transposon Tn4451, at the 5' upstream and...

...coordinates of certain nucleotides are indicated under the corresponding sequence. The nucleotide sequence of chromosomal DNA from N. meningitidis is shown in italics. Two alternative *insertion* sites are indicated by vertical arrows. The two thymidines present in chloramphenicol-susceptible meningococci (strains LNP14371, LNP15339, LNP15345, BM4376, and BM4377), in the transformants, and...

...thymidine at position 92 (N. meningitidis numbering), resulting in the replacement of the conserved phenylalanine by a leucine at position 31 and a 6-bp *insertion* (TCCGGC) resulting in two extra amino acids (serine at position 195 and glycine at position 196) in a highly conserved region of the enzyme. (Ref...transformation and integration of the incoming DNA through homologous recombination between the flanking regions and the corresponding portion of the chromosome of the recipient and *insertion* of the heterologous catP gene. This process would account for the observation that an identical fragment of foreign DNA was *inserted* at the same site in all the transformants...

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S4	15	S3 AND (ANTIBIOTIC? OR GALACTOSIDASE)
S5	15	RD (unique items)

>>>KWIC option is not available in file(s): 399

5/3,K/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13979723 BIOSIS NO.: 200200608544
Transgenic Chlorella as a phytoremedial bioreactor.
AUTHOR: Cohill P R(a); Cannons A C(a)
AUTHOR ADDRESS: (a)University of South Florida, Tampa, FL**USA
JOURNAL: Abstracts of the General Meeting of the American Society for Microbiology 102p354 2002
MEDIUM: print
CONFERENCE/MEETING: 102nd General Meeting of the American Society for Microbiology Salt Lake City, UT, USA May 19-23, 2002
SPONSOR: American Society for Microbiology
ISSN: 1060-2011
RECORD TYPE: Abstract
LANGUAGE: English

...ABSTRACT: incorporates exogenous genetic material and stably expresses it. Exogenous or native genes normally expressed in the nucleus can be isolated and inserted into a transformation *vector* designed specifically for homologous recombination with the chloroplast genome. This *vector* contains intronic DNA sequences found in the ribosomal RNA gene cluster of the chloroplast genome; these intronic sequences surround a gene for streptomycin resistance as well as a *multiple* cloning site. Thus, resistance to this *antibiotic* indicates stable incorporation of the transgenes into the chloroplast genome. Additionally, many *copies* of the chloroplast genome exist in each cell, meaning that overexpression of the transgenes from *multiple* genomic *copies* is possible. One potential transgene expresses phytochelatin synthase (PCS), an enzyme found in plants which produces short peptides, phytochelatins, in response to heavy metal exposure...

...through HPLC analysis, indicating the presence of an active PCS gene in the nuclear genome. The native Chlorella PCS gene can be identified through the *screening* of a cDNA library, and can be compared to the recently isolated wheat and Arabidopsis forms of the gene through sequence analysis. A chloroplast transformation *vector* containing a PCS gene can then be used to *insert* the gene into the Chlorella chloroplast genome, resulting in the overexpression of PCS and optimizing the algal cells as bioreactors that serve to phytoremediate.

5/3,K/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10272449 BIOSIS NO.: 199698727367
Conditionally replicative and conjugative plasmids carrying lacZ-alpha for

cloning, mutagenesis, and allele replacement in bacteria.
AUTHOR: Metcalf William W; Jiang Weihong; Daniels Larry L; Kim Soo-Ki; Haldimann Andreas; Wanner Barry L(a)
AUTHOR ADDRESS: (a)Dep. Biol. Sci., Purdue Univ., W. Lafayette, IN 47907** USA
JOURNAL: Plasmid 35 (1):p1-13 1996
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We describe several new cloning *vectors* for mutagenesis and allele replacement experiments. These plasmids have the R6K-gamma DNA replication origin (oriR-R6Kgamma) so they replicate only in bacteria supplying the II replication protein (encoded by pir), and they can be maintained at low or high plasmid *copy* number by using Escherichia coli strains encoding either wild-type or mutant forms of II. They also carry the RP4 transfer origin (oriT-RP4) so they can be transferred by conjugation to a broad range of bacteria. Most of them encode lacZ-alpha for blue-white color *screening* of colonies for ones with plasmids carrying *inserts*, as well as the f1 DNA replication origin for preparation of single-stranded DNA. Particular plasmids are especially useful for allele replacement experiments because they...

...pir host (via conjugative transfer, transformation, or electroporation), and integrants with the plasmid recombined into the chromosome via homologous sequences are selected using a plasmid *antibiotic* resistance marker. Plasmid-free segregants with an allele replacement can be subsequently selected as Tet-Ss or Suc-R recombinants. A number of additional features (including the presence of *multiple* cloning sites flanked by T3 and T7 RNA polymerase promoters) make these plasmids useful as general cloning *vectors* as well.
MISCELLANEOUS TERMS: ...*ANTIBIOTIC* RESISTANCE MARKER

5/3,K/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10107342 BIOSIS NO.: 199698562260
A versatile low-copy-number cloning vector derived from plasmid F.
AUTHOR: Shi Jianpeng; Biek Donald P(a)
AUTHOR ADDRESS: (a)Dep. Microbiol. Immunol., Univ. Kentucky Med. Center, Lexington, KY 40536-0084**USA
JOURNAL: Gene (Amsterdam) 164 (1):p55-58 1995
ISSN: 0378-1119
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have constructed a cloning *vector* based on plasmid mini-F for use in Escherichia coli. Plasmid pZC320 consists of the ori-2 replication unit of F that confers very low *copy* number (lcn), and includes the sop partition functions to insure stable plasmid maintenance in the absence of selection. A *multiple* cloning site (MCS) containing 16 unique restriction sites is located within the 5' end of the lacZ-alpha gene. Expression of lacz-alpha is under the control of the wild-type lactose operator/promoter (lacOP) region and is efficiently repressed by the lacI repressor. Clones containing *inserts* can be detected using the blue/white *screen* for beta-*galactosidase* (beta-Gal). A T7 promoter allows transcription of cloned *inserts* in the presence of T7 RNA polymerase. We have demonstrated the use of this lcn *vector* for cloning the regulated tetracycline-resistance genes from Tn10, which confer only low-level resistance when present at high *copy* number.

5/3,K/4 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04421186 Genuine Article#: TC459 No. References: 19
Title: A VERSATILE LOW-COPY-NUMBER CLOSING VECTOR DERIVED FROM PLASMID-F
Author(s): SHI JP; BIEK DP
Corporate Source: UNIV KENTUCKY,MED CTR,DEPT MICROBIOL & IMMUNOL/LEXINGTON//KY/40536; UNIV KENTUCKY,MED CTR,DEPT MICROBIOL & IMMUNOL/LEXINGTON//KY/40536
Journal: GENE, 1995, V164, N1 (OCT 16), P55-58
ISSN: 0378-1119
Language: ENGLISH Document Type: ARTICLE (Abstract Available)

Abstract: We have constructed a cloning *vector* based on plasmid mini-F for use in Escherichia coli. Plasmid pZC320 consists of the ori-2 replication unit of F that confers very low *copy* number (Icn), and includes the sop partition functions to insure stable plasmid maintenance in the absence of selection. A *multiple* cloning site (MCS) containing 16 unique restriction sites is located within the 5' end of the lacZ alpha gene. Expression of lacZ alpha is under the control of the wild-type lactose operator/promoter (lacOP) region and is efficiently repressed by the lad repressor. Clones containing *inserts* can be detected using the blue/white *screen* for beta-*galactosidase* (beta Gal). A Tr promoter allows transcription of cloned *inserts* in the presence of T7 RNA polymerase. We have demonstrated the use of this Icn *vector* for cloning the regulated tetracycline-resistance genes from Tn10, which confer only low-level resistance when present at high *copy* number.

5/3,K/5 (Item 1 from file: 315)
DIALOG(R)File 315:ChemEng & Biotec Abs

380695 CEABA Accession No.: 27-03-005120 DOCUMENT TYPE: Journal
Title: A versatile low-copy-number cloning vector derived from plasmid F.
AUTHOR: Biek, D. P.; Shi, Jianpeng
CORPORATE SOURCE: Univ. Kentucky Dept. Microbiol. Immunol. Lexington, KY 40536-0084 USA
JOURNAL: Gene, Volume: 164, Issue: 1, Page(s): 55-58
CODEN: GENED6 ISSN: 03781119
PUBLICATION DATE: 1995 (950000) LANGUAGE: English

ABSTRACT: A cloning *vector*, based on plasmid mini-F, was constructed for use in Escherichia coli. Plasmid pZC320 consists of the ori-2 replication unit of F that confers very low *copy* number and includes the sop partition functions to ensure stable plasmid maintenance in the absence of selection. A *multiple* cloning site containing 16 unique restriction sites is located within the 5' end of the lacZ.alpha. gene. Expression of lacZ.alpha. is under the control of the wild-type lactose operator/promoter (lacOP) region and is efficiently repressed by the lacI repressor. Clones containing *inserts* can be detected using the blue/white *screen* for .beta.-*galactosidase*. A T7 promoter allows transcription of cloned *inserts* in the presence of T7 RNA polymerase.

5/3,K/6 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0309450 DBR Accession No.: 2003-11235 PATENT
Detecting *antibiotic* activity in sample, by contacting sample with transformed Streptomyces coelicolor with promoter derived from vanSREFHAX cluster gene linked to reporter gene producing signal and detecting signal - vector plasmid pGEM-T-mediated gene transfer and expression in Streptomyces coelicolor for use in *antibiotic* screening

AUTHOR: BUTTNER M J; HONG H; PAGET M S B
PATENT ASSIGNEE: PLANT BIOSCIENCE LTD 2003
PATENT NUMBER: WO 2003012128 PATENT DATE: 20030213 WPI ACCESSION NO.: 2003-248185 (200324)
PRIORITY APPLIC. NO.: GB 20021161 APPLIC. DATE: 20020118
NATIONAL APPLIC. NO.: WO 2002GB3372 APPLIC. DATE: 20020723
LANGUAGE: English

Detecting *antibiotic* activity in sample, by contacting sample with transformed Streptomyces coelicolor with promoter derived from vanSREFHAX cluster gene linked to reporter gene producing signal and detecting signal - vector plasmid pGEM-T-mediated gene transfer and expression in Streptomyces coelicolor for use in *antibiotic* screening

ABSTRACT: DERWENT ABSTRACT: NOVELTY - Detecting (M1) in a sample, the activity of an *antibiotic* which affects cell integrity, comprising contacting the sample with a transformed Streptomyces coelicolor microorganism (I) having a nucleic acid encoding a promoter derived from a...

... to a promoter of a gene of the vanSREFHAX cluster, of a heterologous reporter gene capable of causing a detectable signal, or with a heterologous *vector* comprising promoter derived from a gene of the vanSREFHAX cluster of S. coelicolor, operably linked to a heterologous reporter gene capable of causing a detectable signal; (2) producing (M3) an isolated *antibiotic* which affects cell integrity, by performing M1, such as to identify the activity of the *antibiotic* in a sample, and isolating the *antibiotic* from the sample; (3) a transformed microorganism for use in M1, which is transformed S. coelicolor microorganism, which either comprises a heterologous reporter gene capable...

... a detectable signal, which reporter gene is operably linked to a promoter of a gene of the vanSREFHAX cluster, or is transformed with a heterologous *vector* comprising promoter derived from a gene of the vanSREFHAX cluster of (I), operably linked to a heterologous reporter gene capable of causing a detectable signal; (4) a system (II) for detecting an activity of an *antibiotic* in a sample, comprising (I) and a device for detecting the signal produced from the reporter gene; and (5) a kit for performing M1, comprising...

... or observation steps. BIOTECHNOLOGY - Preferred Method: The promoter is derived from vanE = (SC66T3.03); vanF = SC66T3.04), vanH = (SC66T3.05) or vanR = (SCH66.11c). The *antibiotic* is glycolipidic, phosphoglycolipidic, glycopeptidic or peptidic compound which interferes with the physical integrity of the microorganism cell envelope or membrane. The reporter gene encodes a reporter protein which provides the microorganism with resistance to an *antibiotic*, or encodes a reporter protein which can be detected photometrically. The reporter gene is a neo reporter gene. The reporter gene is operably linked to...

... part of the promoter region or operon of the microorganism. The nucleic acid encoding the promoter and the heterologous reporter gene is a plasmid. The *vector* is derived from the neo reporter plasmid, pIJ486. The plasmid is pIJ6883 as given in the specification. (I) further comprises heterologous *copies*, or increased *copy* number, of a gene encoding a trans-acting factor which activates the promoter, and/or a gene encoding a sensor kinase which is receptive to changes in the cell envelope or membrane of the microorganism and activates the trans-acting factor in response to the presence of *antibiotics* which affects cell integrity. The trans-acting factor is vanR (SCH66.11c) and the sensor kinase is vanS (SCH6.10c). The sample is selected from...

...isolate, the product of combinatorial chemical synthesis and the product of combinatorial biosynthesis. The activity is qualitatively correlated with the presence or absence of an *antibiotic*. The activity of the sample is further *screened* for antibiosis of a target organism. (I)

is provided before carrying out M3. Preferred System: In (II), the detector unit is a photosensitive device. (II) is a parallel processing system in which detection of *multiple* activities is assessed using *multiple* cultures of transformed microorganisms. USE - M1 is useful for detecting the activity of an *antibiotic* which affects cell integrity, in a sample (claimed). (I) is useful for *screening* for compounds which have potential as *antibiotics*. EXAMPLE - A 270 base pair DNA fragment carrying the vanE promoter (vanEp0 (SC66T3.03) was generated by the polymerase chain reaction (PCR) using two synthetic...

... primers (vanE1 - 5'-CCCCAAGCTTACACTCAGCAGCTCCAACGCGGT-3'; vanE2 - 5'-CCCCGAATTCTGGTGGCGTTGGCAGCGCTGGTG-3') and genomic DNA of Streptomyces coelicolor M600 as a template. This PCR product was cloned into *vector* pGEM-T (RTM) to create pIJ6882, and the nucleotide sequence of the *insert* was confirmed using standard sequencing procedures to ensure no untended changes had occurred. The vanEp (SC66T3.03) fragment was re-isolated from pIJ6882 as a...

... sites underlined in the oligonucleotide sequences) and cloned into the multicopy promoter probe plasmid pIJ486 cut with EcoRI and HindIII, such that expression of the *vector* aminoglycoside phosphotransferase gene (neo), which confers resistance to both neomycin and kanamycin, depends on vanEp. The resulting plasmid was designated pIJ6883. Plasmid pIJ6883 was introduced...

... and was found not to confer resistance to kanamycin, even to 2 micro-g/ml kanamycin. The vanE (SC66T3.03) promoter was induced by control-*antibiotics* known to target the cell envelope, spores of M600 carrying pIJ6883 were spread on MMT medium minus L-Tyr carrying a lethal concentration of kanamycin...

... disc. The glycopeptides ristocetin and vancomycin and the peptide bacitracin were shown to be potent inducers, as was the glycolipidic compound. In contrast, negative control *antibiotics* that target the ribosome (e.g. thiostrepton, streptomycin) or DNA gyrase (novobiocin), did not induce a halo. The results of the assay for the *antibiotics* used clearly showed that the vanE promoter bioassay detects the cell envelope, thereby allowing the system to act as a *screen* for certain classes of *antibiotic*, such as a broad range of peptidic, glyolipidic and glycopeptidic *antibiotics*, and provided for a genetic *screen* for cell envelope-specific *antibiotics* . The sensitivity of the demonstrated reporter system was inducible and was sensitive to vancomycin at least down to a level of 300 ng. These results clearly showed the use of the system as a *screen*. (29 pages)

DESCRIPTORS: vector plasmid pGEM-T-mediated gene transfer, expression in Streptomyces coelicolor, polymerase chain reaction, appl. *antibiotic* screening bacterium actinomycetes DNA amplification DNA sequence (22, 19)

5/3,K/7 (Item 2 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

0307337 DBR Accession No.: 2003-09122 PATENT

Novel eukaryotic chromosome comprising one or many att sites which permits site-directed integration in the presence of lambda-integrase, useful for site-specific recombination-directed integration of DNA of interest - artificial chromosome-mediated gene transfer and expression in host cell for gene therapy

AUTHOR: PERKINS E; PEREZ C; LINDENBAUM M; GREENE A; LEUNG J; FLEMING E ; STEWART S; SHELLARD J

PATENT ASSIGNEE: CHROMOS MOLECULAR SYSTEMS INC 2002

PATENT NUMBER: WO 200297059 PATENT DATE: 20021205 WPI ACCESSION NO.: 2003-140461 (200313)

PRIORITY APPLIC. NO.: US 366891 APPLIC. DATE: 20020321

NATIONAL APPLIC. NO.: WO 2002US17452 APPLIC. DATE: 20020530

LANGUAGE: English

...ABSTRACT: recombinase that promotes recombination between the sites in the chromosome and in the nucleic acid molecule; (3) a combination (III), comprising (I) and a first *vector* containing the cognate recombination site which recombines with the site engineered into the chromosome; (4) a kit comprising (III), and optionally instructions for introducing heterologous...

... chromosome; (5) introducing (M2) heterologous nucleic acid into a platform artificial chromosome, by: (a) mixing an artificial chromosome comprising a first recombination site and a *vector* comprising a second recombination site and the heterologous nucleic acid; and (b) incubating the resulting mixture in the presence of the recombination protein under conditions...

...comprising an artificial chromosome;(8) introducing heterologous nucleic acid into (V), by: (a) introducing into the cell, platform-ACes having a first recombination site, a *vector* comprising a second recombination site and a heterologous nucleic acid; and (b) incubating the resulting mixture in the presence of a recombination protein under conditions...

... a library ACes, comprising a multiplicity of heterologous recombination sites randomly integrated throughout the endogenous chromosomes; (12) a library of cells (IX) useful for genomic *screening*, comprising multiplicity of cells, where each cell comprises an ACes having a mutually exclusive portion of a chromosomal nucleic acid; (13) making one or more...

... modified iron-induced promoter (IX) comprising a 303 nucleotide sequence, given in the specification; (15) a plasmid or expression cassette (X) comprising (IX); (16) a *vector* (XI) comprising a recognition site for recombination, and a sequence of nucleotides that targets the *vector* to an amplifiable region of a chromosome; (17) *screening* method (M3), involving: (a) contacting a cell comprising a reporter ACes with test compounds or known compounds, where the reporter ACes comprises one or more...

... of the pSR1 plasmid, or their combination. Preferred Combination: (III) further comprises nucleic acid encoding a recombinase, where the nucleic acid is on a second *vector* or on the first *vector*, or on the ACes under an inducible promoter. The *vector* is the plasmid pCXLamIntR or pDsRedN1-attB. Preferred Method: In (M1), the recombinase is chosen from Cre, Gin, Cin, Pin, FLP, a phage integrase and...mutated att recombination site containing at least one mutation that enhances recombinational specificity, a complementary DNA sequence, and an RNA sequence corresponding to it. The *vector* comprising a second site further encodes at least one selectable marker such as a promoterless marker, which, upon recombination is under the control of a...

...marker and the heterologous nucleic acid are transcriptionally linked by the presence of internal ribosome entry site (IRES) between them. The selectable marker is an *antibiotic* resistance gene, and a detectable protein such as chromogenic, fluorescent or capable of being bound by an antibody and fluorescence activated cell sorting (FACS) sorted...

... blue fluorescent protein (BFP) or Escherichia coli histidinol dehydrogenase (hisD). The method involves expressing the heterologous protein and isolating the heterologous protein. In (M2), the *vector* is a PCR product comprising a second recombination site. In (M3), the reporter is operatively linked to a promoter that controls expression of a gene...

... in response to DNA damage induced by apoptosis, necrosis or cell-cycle perturbations, and the test compounds are genotoxicants, and where the unknown compounds are *screened* to assess whether they are genotoxicants. The promoter is a cytochrome P450-profiled promoter, and

the cells are in a transgenic animal and toxicity is...

... nucleic acid within the ACes. Preferred Mutein: (VI) further comprises an amino acid signal for nuclear localization, or an epitope tag for protein purification. Preferred *Vector* : In (XI), the amplifiable region comprises heterochimeric nucleic acid, or rDNA which comprises an intergenic spacer. (XI) further comprises nucleic acid encoding a selectable marker that is not operably associated with any promoter. The *vector* comprises a nucleotide sequence that targets the *vector* to an amplifiable region of a mammalian chromosome or plant chromosome. ACTIVITY - None given. MECHANISM OF ACTION - Gene therapy. No biological data is given. USE... multiplicity of the heterologous recombination sites within the cell's chromosomal DNA, and isolating the multiplicity of ACes, to produce a library of ACes where *multiple* ACes have different portions of the genome within. (XI) is useful for selecting a cell comprising an artificial chromosome (preferably an ACes) which involves introducing...

... into a cell, growing the cells, and selecting cells containing the artificial chromosome that comprises one or more repeat regions. A sufficient portion of the *vector* integrates into a chromosome in the cell to result in amplification of chromosomal DNA. (All claimed.) ADVANTAGE - (II) permits tractable, efficient, rational engineering of the chromosome for a variety of applications. Platform ACes allow *multiple* payload delivery of donor target *vectors* via a positive-selection site-specific, recombination system, and they allow for the inclusion of additional genetic factors that improve protein production and quality. EXAMPLE...

... 538 base pair PCR product homologous to nucleotide 9680-10218 of the human rDNA sequence (GenBank Accession No.U13369) and used as a probe to *screen* a human genomic P1AC (P1 artificial chromosome) library constructed in the *vector* pCYPAC2. Genome systems clone 18720 was isolated in the *screen* and contained three repeats of human rDNA as assessed by restriction analysis. GS Clone 18720, was digested with PmeI, a restriction enzyme unique to a single repeat of the human rDNA (45 kbase), and then religated to form pPACrDNA. The *insert* in pPACrDNA was analyzed by restriction digests and sequence analysis. The pPACrDNA, rDNA sequence were homologous to Genbank Accession U13369, containing an *insert* of 45 kbase comprising a single repeat beginning from the end of one repeat at 33980 (relative to the Genbank sequence) through the beginning of the next repeat upto 35120. Thus, the rDNA sequence was just over 1 *copy* of the repeat extending from 33980 (+/-10 base pairs) to the end of the first repeat (43 kbase) and continuing into the second repeat to...

... Fluorescent in situ hybridization (FISH) analysis was performed on the candidate clones to detect ACes formation. Metaphase spreads from the candidate clones were probed in *multiple* probe combinations. Candidate M2-2d was single cell subcloned by flow sorting and the candidate subclones were reanalyzed by FISH. Subclone 1B1 of M2-2d...

5/3,K/8 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0306331 DBR Accession No.: 2003-08116 PATENT
Novel NF-kappaB-associated polypeptides and polynucleotides useful for diagnosing, treating and preventing cancer, hepatic disorders, aberrant apoptosis, viral infections, autoimmune disorders, asthma and stroke - vector-mediated gene transfer, expression in host cell and antisense oligonucleotide for recombinant protein production, drug screening, vaccine and gene therapy
AUTHOR: CARMAN J; FEDER J; NADLER S
PATENT ASSIGNEE: BRISTOL-MYERS SQUIBB CO 2002
PATENT NUMBER: WO 200286076 PATENT DATE: 20021031 WPI ACCESSION NO.: 2003-093119 (200308)

PRIORITY APPLIC. NO.: US 346986 APPLIC. DATE: 20020109
NATIONAL APPLIC. NO.: WO 2002US12636 APPLIC. DATE: 20020419
LANGUAGE: English

...ABSTRACT: nucleic acid molecule having a nucleotide sequence of only A or T residues; (2) an antibody (Ab) that binds specifically to (I); (3) identifying or *screening* for a compound that modulates the biological activity of NF-kappaB associated molecule; and (4) a compound (C) that modulates the biological activity of a human NF-kappaB associated molecule as identified by the method of (3). WIDER DISCLOSURE - (1) recombinant *vectors* comprising (II); (2) host cells comprising the *vectors* of (1); (3) making the *vectors* of (1) and host cells of (2); (4) T-cell antigen receptors (TCRs) which immunospecifically bind to (I); (5) receptor specific antibodies which prevent ligand...PCR) oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence to synthesize insertion fragments. The primers used to amplify the cDNA *insert* contains BamHI and XbaI restriction sites at the 5' end of the primers corresponding to restriction sites on bacterial expression *vector* pQE-9, in order to clone the amplified product into the expression *vector*. This plasmid *vector* encoded *antibiotic* resistance (Ampr), bacterial origin of replication (ori), isopropyl-B-D-thiogalactopyranoside (IPTG)-regulatable promoter/operato r (P/O), ribosome binding site (RBS), 6-histidine tag (6-His) and restriction enzyme cloning sites. The pQE-9 *vector* was digested with BamHI and XbaI, and the amplified fragment was ligated into pQE-9 *vector* maintaining the reading frame initiated at the bacterial RBS. The ligation mixture was then used to transform the Escherichia coli strain M15/rep4 which contained *multiple* *copies* of the plasmid pREP4, that expressed the lacI repressor and also conferred kanamycin resistance (Kanr). Transformants were identified by their ability to grow on LB...
DESCRIPTORS: ...mellitus, vitamin B12 malabsorption, genetic syndrome, Huntington chorea, Turner syndrome, bacterium infection, cardiovascular disorder, infertility, psoriasis, hemolytic anemia therapy, diagnosis, prevention, gene therapy, immunophenotyping protein *antibiotic* -resistance RNA enzyme hybridization DNA amplification tumor leuko virus retro virus AIDS lenti virus hepadna virus flavi virus herpes virus orthomyxo virus DNA sequence protein...

5/3,K/9 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0304404 DBR Accession No.: 2003-06189 PATENT
Novel DNA molecule for producing filamentous phages, has filamentous phage DNA into which recombinant DNA with multiple cloning site having a series of restriction sites that do not appear in phage DNA, is inserted - vector-mediated gene transfer and expression in host cell for recombinant protein production and phage display
AUTHOR: GERSHONI J M; ENSHEL D
PATENT ASSIGNEE: UNIV RAMOT APPLIED RES and IND DEV LTD; MCINNIS P 2002
PATENT NUMBER: WO 200281635 PATENT DATE: 20021017 WPI ACCESSION NO.: 2003-058517 (200305)
PRIORITY APPLIC. NO.: US 281401 APPLIC. DATE: 20010405
NATIONAL APPLIC. NO.: WO 2002US10720 APPLIC. DATE: 20020405
LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A DNA molecule (I) comprising a filamentous phage DNA (Ia) into which a recombinant DNA sequence (Ib) containing a *multiple* cloning site (MCS) having a series of restriction sites that do not appear in (Ia), has been inserted, is new. (Ib) is designed and inserted...

...different random polypeptide as a polypeptide of interest; and (3) a DNA construct (IV) for insertion into the DNA of a filamentous phage, comprising a *multiple* cloning site comprising a series of restriction

sites that do not otherwise appear in the DNA of the phage into which the construct is intended...

... into which the construct is intended to be inserted. BIOTECHNOLOGY - Preferred Molecule: A positive selection marker (e.g. tetracycline resistance gene) is present in the *multiple* cloning site, between the native and recombinant pVIII genes. An additional bacteriophage gene is also present in MCS. The additional bacteriophage gene is a second...

... not appear anywhere else in the DNA molecule. (IV) further includes a positive selection marker and a second pVIII gene. USE - (II) is useful for *screening* for a molecule which binds to a peptide of interest, by bringing a molecule to be *screened* into contact with (II) which expresses the peptide of interest, and if the molecule binds to the peptide of interest, identifying and producing the molecule. (III) is useful for *screening* for peptides which bind to a molecule of interest, by bringing the molecule of interest into contact with (III), identifying any peptide expressed by a...

... pVIII chimeric proteins in mosaic bacteriophages. (II) is useful for expression and display of combinatorial random peptides. ADVANTAGE - (I) is genetically stable and has high *copy* number, and thus allows production of type 88 phages displaying a peptide of interest with genetic stability and high *copy* number. (I) also produces high titers of recombinant phages. EXAMPLE - The ftac88 *vector* was constructed as follows. First, the recombinant pVIII gene, designated pVIIISTS was generated by introducing a 62 base pair *insert* three codons downstream to the leader peptide using the SOEing polymerase chain reaction (PCR) mutagenesis. For this, four oligonucleotides were used. ON1 and ON2 were...

...ON4 were used to PCR generate from a 899 base pair fragment. ON2 and ON3 each contained 5'-extension corresponding to the 62 base pair *insert*. Thus, the resulting PCR fragments contained an identical 30 base pair stretch of the novel sequence. The two fragments were purified from agarose gel, mixed...added adenine to the 3' ends of PCR products and thus the resulting 1071 base pair fragment was directly ligated with the linearized pGEM-T *vector* containing complementary 3' thymidine overhangs, generating the pGEM-T (p8STS) construct. Next, the wild type pVIII gene was PCR amplified using the oligonucleotides ON1 and...

... blunt-ended SnaBI linearized pGEM-T (p8STS) construct generating the pGEM-T (p88STS) construct. To remove the SnaBI site in tetR gene, the fd-tet *vector* was digested with XbaI and BstXI restriction enzymes and the 8458 base pair fragment was purified from agarose gel. The purified fragment was ligated with...

DESCRIPTORS: ...site, tetracycline-resistance marker gene transfer, expression in host cell, polymerase chain reaction-mediated mutagenesis, agarose gel electrophoresis, appl. phage display, combinatorial peptide library construction *antibiotic*-resistance DNA amplification surface display (22, 11)

5/3,K/10 (Item 5 from file: 357)

0292919 DBR Accession No.: 2002-14766 PATENT

New human cadherin-like asymmetry protein(s) (CLASP)-2 for modulating an immune response, and for treating multiple sclerosis, rheumatoid arthritis, endometriosis, lupus, autoimmune thyroiditis, septic shock, and sepsis - vector-mediated gene transfer and expression in host cell, antisense oligonucleotide, agonist, antagonist, monoclonal antibody, DNA primer, DNA probe, antisense, ribozyme and transgenic animal model construction for use in drug screening, gene therapy and forensics

AUTHOR: LU P S

PATENT ASSIGNEE: ARBOR VITA CORP; GARMAN J D; CANDIA A F 2002

PATENT NUMBER: WO 200231117 PATENT DATE: 20020418 WPI ACCESSION NO.: 2002-416861 (200244)
PRIORITY APPLIC. NO.: US 687837 APPLIC. DATE: 20001013
NATIONAL APPLIC. NO.: WO 2001US32202 APPLIC. DATE: 20011015
LANGUAGE: English

...ABSTRACT: to or exactly complementary to NS; (2) an isolated CLASP-2 polynucleotide (III) comprising a nucleotide sequence that is 90 % identical to NS; (3) a *vector* (IV) comprising (II); (4) an expression *vector* comprising (II) in which the nucleotide sequence of the polynucleotide is operatively linked with a regulatory sequence that controls expression of the polynucleotide in a...

... probes for CLASP-2 expression), as a lymphocyte marker and for therapeutic purposes. CLASP-2 polynucleotides can construct transgenic and knockout animals, e.g., for *screening* of CLASP-2 agonists and antagonists. CLASP-2 polynucleotides can *screen* for CLASP-2 agonists and antagonists. CLASP-2 polynucleotides are can detect CLASP-2 expression in cells and can diagnose diseases or disorders (e.g... lymphopenia, thrombocytopenia, or hemoglobinuria) CLASP-2 polynucleotides or polypeptides can treat or detect autoimmune diseases, e.g., Addison's disease, hemolytic anemia, Grave's disease, *multiple* sclerosis, rheumatoid arthritis, lupus, endometriosis, autoimmune thyroiditis, and autoimmune pulmonary inflammation. CLASP-2 can be used to treat anaphylaxis or hypersensitivity to an antigenic molecules...

... treat or detect infectious agents. The presence or absence of hCLASP-2 nucleotide and amino acid sequences in a biological sample can be used in *screening* assays as medical diagnostics to aid in clinical decision-making e.g., hCLASP-2-based diagnostics involves *screening* assays for vaginal bleeding of unknown cause. CLASP-2 polynucleotides are used as new chromosome marking reagents. The CLASP-2 polynucleotides can identify individuals from...

... CLASP-1 sequences. IMAGE clone 815795 was sequenced completely. A polynucleotide probe prepared from the 815975 sequence was labeled with 32P-dCTP and used to *screen* human cDNA libraries including Jurkat and Ramos B cell cDNA library. Several clones were identified and clone C9, with an *insert* of 3752 base pairs, was sequenced. A probe was prepared from the C9 sequence and used to rescreen the cDNA libraries. Several clones were isolated, but could not be excised from the phage without deleting the *insert*. To circumvent this problem, an anchor polymerase chain reaction (PCR) was performed using an M13F primer and a CLASP-2 primer (C96A5). The PCR fragment...

... additional but incomplete cDNA sequence and was determined to carry a mutation that may have allowed it to be propagated in bacteria. Commercial libraries from *multiple* tissue sources including human placenta, B cell, T cell and peripheral blood were exhaustively *screened* and re-*screened* resulting in the acquisition of only partial cDNAs. Generation of cDNA libraries using oligo dT or CLASP-specific primers also resulted in the acquisition of partial cDNAs. Genomic libraries were *screened* to obtain a portion of the genomic locus for each of the CLASP genes, and a genomic walk was initiated to obtain 5 exons and...

... sequencing) and shown to be additional human CLASP-2 5 sequence. Standard isolation of some of the CLASP cDNAs from a pure phage population following *screening* of commercially available cDNA libraries resulted in no bacterial colonies. Similarly, certain RT-PCR products could not be cloned into standard plasmid *vectors*. No colonies were isolated by cloning these fragments into *vectors* lacking promoters, reverse orientations, low *copy* *vectors*, or by growth at altered temperatures or levels of *antibiotic* for plasmid selection. To circumvent these problems direct sequencing of RT- PCR products was performed. In-frame stop codons were not present

suggesting that the...

5/3,K/11 (Item 6 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0287801 DBR Accession No.: 2002-09648 PATENT
Transposon-mediated multiplex sequencing of DNA comprises cloning multiple DNA target sequences into a vector, and randomly inserting selectable transposons with sequencing primers into the DNA target-containing vectors - vector expression in host cell, polymerase chain reaction and selectable marker for DNA sequencing and desired vector detection
AUTHOR: AUGUST P R; KEAGLE P J; LONG H; WIENCIS A; CALL K; DRAPER M
PATENT ASSIGNEE: AVENTIS PHARM INC 2002
PATENT NUMBER: WO 200204674 PATENT DATE: 20020117 WPI ACCESSION NO.: 2002-171726 (200222)
PRIORITY APPLIC. NO.: US 216381 APPLIC. DATE: 20000707
NATIONAL APPLIC. NO.: WO 2001US21269 APPLIC. DATE: 20010705
LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A process for parallel transposon-mediated sequencing of DNA, comprising cloning *multiple* DNA target sequences into a *vector* , and inserting selectable transposons with sequencing primers randomly into the DNA targeting-containing *vectors* , is new. DETAILED DESCRIPTION - A process for parallel transposon-mediated sequencing of DNA, is new, and comprises: (a) providing one or more target DNA sequences, each inserted into a *vector* at an insertion site; (b) amplifying each target DNA-containing *vector* and pooling them, where each target DNA sequence is represented in the pool in an equal amount per kbase; (c) exposing the pool of target DNA-containing *vectors* to a selectable transposon, which integrates into the target DNA-containing *vectors* at random sites to form a pool of target DNA- and transposon-containing *vectors* ; (d) transforming cells with the pool of target DNA- and transposon-containing *vectors* , and isolating and growing the a representative number of individual transformants into cultures under selection conditions; (e) performing polymerase chain reaction (PCR) on DNA from each culture using a pair of primers complementary to the 3' ends of the *vector* sequence at the insertion site and has an extension time during each reaction cycle sufficient to efficiently produce a full-length *copy* of the *vector* sequence but too short to efficiently produce a full-length *copy* of the *vector* sequence with the transposon inserted; (f) measuring the amount of DNA produced in each PCR; and (g) sequencing the transposon flanking regions of the target DNA-containing *vectors* from those cultures corresponding to the PCR, which produced substantial amounts of DNA. BIOTECHNOLOGY - Preferred Process: The steps of the process are automated. The amount ...
... dsDNA to the finished reaction and measuring the resulting amount of fluorescence. The fluorescent stain selective for dsDNA is PICOGREEN (RTM) or bisbenzimide dyes. The *vector* and the transposon carry different selectable markers, where the transposon is at least 3 kbase, preferably 4 or 5 kbase. The transposon is GPS-Apra-2um-URA3. The transposon is equal to or greater than the *vector* in length, or at least 5/4 the length of the *vector*. When the transposon is 5 kbase, the *vector* is 4 kbase. The transposon may be at least 5/3 the length of the *vector*, where when the transposon is 5 kbase, the *vector* is 3 kbase. USE - The method is useful for multiplex sequencing of DNA and for distinguishing the desired constructs with transposons inserted into the target...

... constructs with transposons inserted elsewhere. ADVANTAGE - The new method is faster, more efficient, inexpensive, and can be easily automated. The method provides a quick PCR *screen* that allows for the elimination of unproductive transformants that have the transposon inserted into the *vector* sequence and would yield mainly *vector*

sequences if sequenced. The method prevents waste of time and resources in performing reactions sequencing the *vector* instead of the DNA fragments of interest, and minimizes the amount of non-target DNA sequence generated. The process does not rely on any particular *vector* and does not require any re-cloning steps, and sequencing of the *vector* backbone is minimized or eliminated with the *screening* step. The use of a pool of target DNAs allows a single transposon reaction and a single transformation to be performed, instead of requiring one...

... of that, with priming sties at each end, being randomly inserted into the target DNA. Polymerase chain reaction (PCR) amplification of the 3.0 kbase *vector* using complement M13F and complement m13R primers, 2 distinct PCR products could be visualized on a slab gel. A 3.0 kbase PCR product indicates that the transposon must be in the target DNA sequence since the *vector* is 3.0 kbase. A 4.7 PCR product indicates a *vector* (3.0 kbase) containing the transposon (1.7 kbase). Thus, clones containing the transposon in the target DNA sequence could be identified by the presence of 3.0 kbase PCR products on the gel. Clones containing the transposon in the *insert* were identified by selecting colonies from the transformation following the transposon reaction, inoculating them on media containing both *antibiotics* for selection, apramycin, and the plasmid *antibiotic*. Culture was grown overnight and PCR was performed the following day by kerplunking about 1 micro-l of culture into a PCR cocktail in a...

...PCR, 8 micro-l of the PCR reaction was subjected to gel electrophoresis. There was clearly detectable size distinction between the PCR products from the *vector* sequence without the transposon and *vector* sequence including the transposon. (31 pages)

5/3,K/12 (Item 7 from file: 357)

0283403 DBR Accession No.: 2002-05250 PATENT

Detecting activity of an *antibiotic* e.g. penicillins, glycopeptides in a sample, by using a system which comprises a promoter regulated by a two-component signal transduction system, linked to a reporter gene - plasmid pIJ5953 expression in host cell for producing *antibiotic*

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PATENT ASSIGNEE: PLANT BIOSCIENCE LTD 2001

PATENT NUMBER: WO 200192559 PATENT DATE: 20011206 WPI ACCESSION NO.: 2002-114359 (200215)
PRIORITY APPLIC. NO.: GB 200013384 APPLIC. DATE: 20000601
NATIONAL APPLIC. NO.: WO 2001GB2400 APPLIC. DATE: 20010530
LANGUAGE: English

Detecting activity of an *antibiotic* e.g. penicillins, glycopeptides in a sample, by using a system which comprises a promoter regulated by a two-component signal transduction system, linked to a reporter gene - plasmid pIJ5953 expression in host cell for producing *antibiotic*

ABSTRACT: DERWENT ABSTRACT: NOVELTY - Detecting (I) activity of *antibiotic* affecting cell integrity in sample, comprising contacting sample with a transformed microorganism having a nucleic acid encoding a promoter (P) linked to reporter gene (RG...

... which is upregulated by a two-component signal transduction system and observing the microorganism for the signal, is new. DETAILED DESCRIPTION - Detecting (I) activity of *antibiotic* affecting cell integrity in sample, comprising: (a) contacting the sample with a transformed microorganism which comprises a nucleic acid encoding (P) operably linked to a...

... P). INDEPENDENT CLAIMS are also included for the following: (1) producing (II) a transformed microorganism for use in (I) by transforming a microorganism with a *vector* comprising (P) operably linked to a heterologous RG capable of causing a detectable signal; (2)

a transformed microorganism (III) for use in (I), which is transformed with a *vector* comprising (P) operably linked to a heterologous RG capable of causing a detectable signal; (3) a system (IV) for detecting activity of an *antibiotic* in a sample, comprising (III) and a device for detecting the signal produced from RG; and (4) a kit for performing (I), comprising a preparation...

... and VanR which regulate the vanH promoter, or from KdpD and KdpE. RG encodes a reporter protein which provides the microorganism with resistance to an *antibiotic* and which can be detected photometrically. The nucleic acid is in the form of an extrachromosomal *vector* and (P) encoded by the nucleic acid is native to the microorganism or the species of the microorganism. Some or all of the genes encoding the two component system are present at enhanced *copy* number in the transformed microorganism relative to the corresponding untransformed microorganism, and are operably linked to a strong promoter to enhance their concentration in the microorganism. The microorganism is selected to have an enhanced sensitivity to cell wall-specific *antibiotics* . The microorganism is a bacterium, preferably Streptomyces or Mycobacteria. The sample is a culture supernatant, a soil isolate, the product of combinatorial chemical synthesis or combinatorial biosynthesis. In (II), the *copy* number of the genes encoding the two component system is enhanced in the transformed microorganism relative to the corresponding untransformed microorganism. Preferred System: In (IV), the device for detecting the signal is a photosensitive signal. (IV) is a parallel processing system in which detection of *multiple* activities is assessed using *multiple* cultures of transformed microorganisms. USE - (I) is useful for detecting the activity of an *antibiotic* which affects cell integrity in a sample, which is qualitatively correlated with the presence or absence of an *antibiotic*. The activity of the sample is further *screened* for antibiosis of a target organism. Thus the method is useful for producing an isolated *antibiotic* which affects cell integrity. (All claimed). (I) is useful for detecting activity of penicillins (such as penicillin G, amoxycillin and ticarcillin), glycopeptides (such as teicoplanin...

... base pair BglII fragment and cloned into the multicopy promoter probe plasmid pIJ486 Ward et al., 1986 cut with BamHI, such that expression of the *vector* aminoglycoside phosphotransferase gene (neo), which confers resistance to both neomycin and kanamycin, depends on sigEp. The orientation of the *insert* was determined by digestion with SphI. The resulting plasmid was designated pIJ6880. The plasmid was introduced by protoplast transformation into M600 Chakraburtty and Bibb, 1997...

... coelicolor A3(2), and was found to confer resistance to 80 micro-g/ml kanamycin. To investigate if the sigE promoter was induced by control-*antibiotics* known to target the cell envelope, spores of M600 carrying pIJ6880 were spread on MMT medium (Katz et al., 1983) carrying a lethal concentration of...

... growth around the disc. The glycopeptides teicoplanin, ristocetin and vancomycin (and the peptide bacitracin, data not shown) were particularly potent inducers. In contrast, negative control *antibiotics* that target the ribosome (e.g. thiostrepton, streptomycin) or DNA gyrase (novobiocin) did not induce a halo. Thus it was clear that this sigE promoter bioassay detected structurally unrelated *antibiotics* with varied targets in the cell envelope, allowing the system to provide a broad-range, generic *screen* for cell envelope-specific *antibiotics*.(29 pages)

DESCRIPTORS: vector plasmid pIJ5953 expression in Streptomyces sp., Mycobacterium sp., culture medium, promoter, reporter gene, component system, appl. *antibiotic*, e.g. benzypenicillin, amoxycillin, ticarcillin, glycopeptide,teicoplasnin, ristocetin, vancomycin, peptide etc. isol. affecting cell integrity act. det., drug screening gene transfer bacterium actinomycetes *antibiotic* arene C-amide lactam

C-acid penicillin het-N het-NO bridge-struct. macrocycle phenol-ether phenol alcohol chlorine peptide glycoside amine (Volume 21, Issue...
SECTION: PHARMACEUTICALS-*Antibiotics*-

5/3,K/13 (Item 8 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0112550 DBR Accession No.: 91-00192
Molecular cloning and expression of an immunodominant 53-kDa excretory-secretory antigen from Trichinella spiralis muscle larvae - gene cloning in Escherichia coli; DNA sequence; DNA probe and recombinant antigen preparation for potential application in trichinellosis diagnosis
AUTHOR: Zarlenga D S; Gamble H R
CORPORATE SOURCE: U.S. Department of Agriculture, ARS, Biosystematic Parasitology Laboratory, LPSI, Bldg. 1180, BARC-East, Beltsville, MD 20705, USA.
JOURNAL: Mol.Biochem.Parasitol. (42, 2, 165-74) 1990
CODEN: MBIPDP
LANGUAGE: English

ABSTRACT: A Trichinella spiralis muscle larva cDNA gene bank was constructed in Escherichia coli Y1089, using phage lambda-gt11 as *vector*. Phage TsA-12 was selected by *screening* with pig and rabbit antibodies to the T. spiralis excretory-secretory (ES) antigen. The positive clone contained an *insert* 539 bp in length, encoding a mol.wt. 123,000 beta-*galactosidase* (EC-3.2.1.23) fusion protein which did not cross-react with Trichuris suis or Ascaris suum infection serum. The DNA sequence of the *insert* was determined. The TsA-12 *insert* hybridized to mRNA sequences expressed in both muscle larva and adult stages of T. spiralis, but concomitant expression of the native antigen was not observed...

... adult ES products. Southern blot hybridization with radiolabeled TsA-12 DNA probes verified TsA-12 as a T. spiralis-specific sequence that was present in *multiple* *copies* within the parasite genome. The recombinant antigen could be used in an ELISA test to detect antibodies to T. spiralis in experimentally infected mice as...

5/3,K/14 (Item 9 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0059163 DBR Accession No.: 87-03511
Phage lambda and plasmid expression vectors with multiple cloning sites and lacZalpha-complementation - construction and characterization
AUTHOR: Windle B E
CORPORATE SOURCE: Molecular Biology and Virology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA.
JOURNAL: Gene (45, 1, 95-99) 1986
CODEN: GENED6
LANGUAGE: English

ABSTRACT: 2 New phage lambda *vectors* , lambda-DL10 and lambda-DL11, contain the alpha-complementating fragment of lacZ and *multiple* cloning sites found in the polylinker region of M13mp10 and M13mp11, respectively. The *vectors* provide *multiple* restriction sites for insertion of foreign DNA and a simple *screen* for *inserts* on 5-bromo- 4-chloro- 3-indolyl- beta-D-galactoside plates. Genes that are lethal when expressed from a plasmid can be cloned, and a...

... expression of foreign genes. Fusion proteins can be made with a minimum contribution from the leading lacZ peptide, and expression from 1 or very few *copies* of a cloned gene when integrated into the Escherichia

coli chromosome can be analyzed. Plasmids pPR110 and pPR111, constructed from lambda-DL10 and -DL11 and pCQV2, provide a means to clone genes into *multiple* cloning sites directly downstream of the strong lambda PR promoter under the control of the cI857 repressor. These plasmids allow proteins fusions to be made...
DESCRIPTORS: phage lambda-DL10, phage lambda-DL11, plasmid pPR110, plasmid pPR111 construction, characterization, appl. as cloning vector, beta-*galactosidase* lacZ gene alpha-complementation enzyme EC-3.2.1.23

5/3,K/15 (Item 1 from file: 370)

00500975 (USE 9 FOR FULLTEXT)
NF-AT-Driven Interleukin-4 Transcription Potentiated by NIP45
Hodge, Martin R.; Chun, Hyung J.; Rengarajan, Jyothi; Alt, Aya; Lieberson, Rebecca; Glimcher, Laurie H.
Department of Cancer Biology, Harvard School of Public Health and Department of Medicine, Harvard Medical School, Boston, MA 02115, USA.
Science Vol. 274 5295 pp. 1903
Publication Date: 12-13-1996 (961213) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2627

(THIS IS THE FULLTEXT)

...Text: tissue-specific IL-4 expression (B3) . c-Maf acts in synergy with NF-AT proteins to transactivate the IL-4 promoter. The inducible expression of *multiple* cytokine genes and cell surface proteins after T cell receptor stimulation requires members of the NF-AT transcription factor family (B1) (B4) (B5) (B6...
...B10) . One class of interactors encoding a fusion protein with apparently high affinity for the NF-ATp(RHD)-Gal4 bait, as exhibited by high (beta) -*galactosidase* activity and ability to confer leucine prototrophy, was isolated. The interaction with this factor was specific, because no interaction was detected with baits that encoded...

...The ability of this polypeptide to interact specifically with NF-ATp in vivo was tested in mammalian cells. The 1.9-kb *insert* was subcloned into a mammalian expression *vector* that fused the coding region to an epitope tag from an influenza hemagglutinin (HA) peptide. This tagged construct was cotransfected with an NF-ATp expression...

...Because the transcript size detected by northern blot analysis (below) was approximately 3.1 kb, the 1.9-kb *insert* from this clone was used to *screen* a T cell cDNA library to identify full-length clones. *Screening* of a library containing approximately 8 x 10.sup(5) clones yielded an isolate that contained an additional segment of 180 bp located 868 bp... transactivates NF-AT-driven transcription in this cell line in the absence of exogenous stimulation (B12) . The cDNA encoding NIP45 was cloned into an expression *vector* and cotransfected into HepG2 cells with NF-ATp and a reporter gene containing tandem *copies* of the NF-AT binding site (3XNF-AT-CAT). Transfection of NIP45 alone into HepG2 cells with the 3XNF-AT-CAT reporter did not lead...

...NIP45 cannot act on its own to transactivate an NF-AT target sequence. Overexpression of NF-ATp alone resulted in substantial (six times greater than *vector* control) transactivation of the NF-AT-CAT reporter (Fig. 4A) (B12) . Cotransfection of NIP45 plus NF-ATp resulted in a four to five times increase in CAT activity relative to transfection with NF-ATp alone and a 25 to 30 times increase over that seen with *vector* alone (Fig. 4A). This increase was not observed when a mutant 3XNF-AT-CAT reporter or a control major histocompatability complex (MHC) class II promoter...

...ATc, NF-AT3 or NF-AT4 plus the NF-AT-CAT reporter plasmid. All NF-AT family members can transactivate a reporter gene containing three *copies* of an NF-AT-AP1 site when overexpressed in HepG2 cells, although to different extents (Fig. 4A). Although NF-ATp was the most potent transactivator...B lymphoma cells stably transfected with NF-ATp and c-maf expression *vectors* produce low amounts of endogenous IL-4 protein (B3) . In contrast, endogenous IL-4 is not detected upon transient transfection, which presumably reflects the lower...

...to levels detectable in a transient expression system. Therefore, M12 cells were transiently cotransfected with c-maf and NF-ATp and either NIP45 or control *vector*. Four independent sets of transient transfections were done and assayed for secretion of IL-4 into the culture supernatant. For each set of transfections, inclusion...

...Interacting Protein (NIP45). (A) Interaction of NIP45 and NF-ATp in HepG2 cells: cDNA from the NIP45 plasmid was cloned into the epitope tag expression *vector* pCEP4-HA (B16) . This construct results in the in-frame fusion of amino acids with the sequence YPYDVPDYA (B17) of the influenza hemagglutinin protein to...

...Lysates were prepared from HepG2 cells transfected with NIP45-HA, NF-ATp, or both plasmids, as indicated. Samples indicated with (-) indicate cotransfection with corresponding expression *vector* (for NF-ATp) or an out of frame fusion with the epitope tag (NIP45-HA). Top and middle, lysates were immunoprecipitated with antibody to NF...IL-4 promoter. HepG2 cells were transfected with an IL-4-CAT reporter construct (extending to -732 bp of the IL-4 promoter) and expression *vectors* or controls for NIP45, NF-ATp, and c-maf as indicated. The control for NIP45 was a frame shift mutant at amino acid 13. Controls for NF-ATp and c-maf were the empty expression *vectors* pREP4 and pMEX, respectively (B3) . Representative CAT assays and bar graphs are depicted as in (A...
...endogenous IL-4 production. M12 B lymphoma cells were transiently cotransfected with expression plasmids for NF-ATp and c-maf together with NIP45 or pCI *vector* control, and the concentration of IL-4 in supernatants harvested 72 hours later was measured by enzyme-linked immunosorbent assay (ELISA) (B21...